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THE RELATION OF SOIL MOISTURE AND NITRATES TO THE EFFECTS OF SOD ON APPLE TREES

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CONTENTS

IE RELATION OF SOIL MOISTURE AND NITRATES TO THE EFFECTS OF SOD ON APPLE TREES

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It has frequently been noted that the continuous growth of grass ıder apple trees exerts an injurious effect on the growth of the trees. 'hen subjected to this condition on certain soils, the trees grow more owly and have not the healthy appearance that distinguishes trees own on tilled soil either with or without a cover crop. The injurious fluence of the grass has been attributed to several causes. Hedrick 909 and 1914), and also Woodbury, Noyes, and Oskamp (1917), have awn attention to the importance of soil moisture for tree growth and it production, and they present data to show that the moisture ntent of the soil under sod is less at certain times of the year than under ltivation. Bedford and Pickering (1911), after long and careful experi- entation on the soil at the Woburn Experimental Fruit Farm, concluded at, in that soil at least, the growth of grass produced a toxic substance at was a direct poison to the trees. Lyon and Bizzell (1913) have suggested t the lack of sufficient available nitrogen, due to the property that ss appears to possess of causing an almost complete disappearance of rates in the soil, might account for the injury to the trees. It was h the purpose of testing the last of these hypotheses that the exper- nt herein described was begun.

PLAN OF THE EXPERIMENT

Thirty plats of land, each 43.6 feet long and 10 feet wide, or $\frac{1}{100}$ in size, were used for the experiment. There was a 2-foot space een adjacent plats, and each plat had a tile drain on one side. nty apple trees were planted on each plat, the trees being set 4 feet t each way. There was also a row of trees on the spaces between s, and these trees were 4 feet from all adjacent trees. These were used in measuring the effect of the treatment given to the soil. The s used for the experiment were one-year-old whip-grafted McIntosh k varying from 2 to 4 feet in height. They were selected from a ery on the university farm at Ithaca, and as soon as removed from soil they were distributed into thirty lots, each of which finally sted of twenty specimens of approximately the same size and . One tree from every lot was planted on each of the thirty plats. there was obtained at the beginning of the experiment, for each e various treatments, a uniform average vigor with about the coefficient of variability. The planting was done from April 27 to 2, 1917.

As soon as they were planted, all the trees were cut back to within 4 inches of the ground. Only one sprout was permitted to grow to form the trunk, but no other pruning was given during the course of the experiment. Precautions were taken to prevent damage from diseases, insects, and rodents, but nevertheless a few trees had to be discarded because of injury.

The plats were planted alternately to timothy and to rye. The timothy was maintained continuously throughout the experiment on the plats on which it was planted. It was cut several times in a season and was allowed to rot as it lay. The rye was planted each year about the middle of August, and was turned under as early in the spring as the condition of the soil would permit. During the spring and early summer, the soil was stirred with a spring-tooth cultivator and all weeds were eradicated.

Each plat was fertilized with acid phosphate at the rate of 450 pounds to the acre, and with muriate of potash at the rate of 240 pounds. This was done each spring just after spading under the rye. In addition, each plat was limed previous to the beginning of the experiment. The main feature of the investigation was to ascertain the effect of applications of various quantities of nitrate of soda on the growth of the trees and on the nitrate content of the soil. Equal quantities of nitrate of soda were applied to pairs of timothy and cultivated plats in amounts of 100, 300, and 900 pounds to the acre, while some plats received no nitrate. Each treatment was repeated on four plats, with the exception of two which were in triplicate owing to the fact that only thirty plats were available. The treatments and the plat numbers are shown in table 1:

TABLE 1. CROPPING TREATMENTS, AND APPLICATIONS OF NITRATE OF SODA

Plat	Cropping treatment	Nitrate of soda applied (pounds per acre)	Plat	Cropping treatment	Nitrate of soda applied (pounds per acre)
1001	Timothy sod continuously	0	1101	Timothy sod continuously	300
1002	Timothy sod continuously	100	1102	Timothy sod continuously	900
1003	Cultivation and rye	100	1103	Cultivation and rye	900
1004	Timothy sod continuously	300	1104	Timothy sod continuously	0
1005	Cultivation and rye	300	1105	Cultivation and rye	0
1006	Timothy sod continuously	900	1106	Timothy sod continuously	100
1007	Cultivation and rye	900	1107	Cultivation and rye	100
1008	Timothy sod continuously	0	1108	Timothy sod continuously	300
1009	Cultivation and rye	0	1109	Cultivation and rye	300
1010	Timothy sod continuously	100	1110	Timothy sod continuously	900
1011	Cultivation and rye	100	1111	Cultivation and rye	900
1012	Timothy sod continuously	300	1112	Timothy sod continuously	0
1013	Cultivation and rye	300	1113	Cultivation and rye	0
1014	Timothy sod continuously	900	1114	Timothy sod continuously	100
1015	Cultivation and rye	900	1115	Cultivation and rye	100

The quantities of nitrates present in the soil of each plat were deter-ined in 1919 and 1920 before spading under the rye in the spring, and intervals thereafter until the plats were planted in summer. Another termination was made late in the autumn of each year. These analyses rve as a guide to show whether the nitrate added persisted in the soil whether it disappeared.

METHODS USED FOR MEASURING THE EFFECT OF THE TREATMENTS ACCORDED THE SOIL

The effect of timothy as compared with a cover crop, and of the veral quantities of nitrate of soda, on the soil and on the tree growth as measured, as has been explained, by determining from time to me the moisture and nitrate content of the soil of each plat, the cir-umference of the tree trunks, and the green weights of all the trees hen dormant and of most of the roots. A description follows of each f the methods used in making these measurements.

Determination of moisture and nitrates in soil

Samples were taken to a depth of 8 inches, with a 1½-inch auger. ne borings were made on each plat, making a rate of one boring to ch 48.3 square feet. A composite was made of the nine borings from ch plat, and the portions for moisture and nitrate determinations were thdrawn after remixing in the laboratory.

Determinations of moisture were made by drying 100 grams of soil constant weight at the temperature of boiling water. Nitrates were termined by extracting the soil with five parts of distilled water, tering thru a Pasteur-Chamberland filter, and using the phenol-sulfonic acid method for the remainder of the process. In expressing trate nitrogen in pounds per acre, the weight of an acre eight inches of l is assumed to be 2,500,000 pounds.

Measurement of tree growth

After each season's growth, the circumference of the trees was asured at a marked region about two inches below the origin of the trunk. In the spring of 1921, before the buds began to swell, the s of the trees were cut as close to the surface as possible and immedi-ly weighed. The girth records were also checked at this time by asurements of the diameter of the annual rings. The heights of the es and the spread of the branches were recorded in the nearest tenth a foot.

The soil with the sod or cover crop was not disturbed until the summer of 1921. In the meantime, the few sprouts that came from the stubs were removed as soon as they appeared. When the trees were dug, care was taken to remove all roots larger than ⅛ inch in diameter. The roots were heeled in until they were to be weighed, so that they would lose no moisture. The total weight of the trees therefore represents the weight of the top, determined in the dormant condition in spring, plus the weight of the roots, determined several months later. The underground part of the trunk was cut from the root system at the graft union, and its weight was added to that of the top.

MOISTURE IN THE SOIL

The moisture content of the soil, as shown from time to time by the analyses made in 1919 and in 1920, is recorded in tables 2 and 3, respectively:

TABLE 2. Moisture Content in Surface Eight Inches of Soil in 1919*
(Averages for all plats receiving the same treatment)

Plats	Crop	Nitrate of soda applied (pounds per acre)	Moisture (per cent in dry soil)			
			April 22	July 8	August 15	October [illegible]
1001, 1008, 1104, 1112	Sod	0	27.8	17.1	17.8	18.[illegible]
1009, 1105, 1113	Cultivation	0	27.6	19.9	16.0	[illegible]
1002, 1010, 1106, 1114	Sod	100	28.2	16.8	18.4	19.0
1003, 1011, 1107, 1115	Cultivation	100	27.5	20.4	16.8	13.[illegible]
1004, 1012, 1101, 1108	Sod	300	28.2	16.8	18.0	17.[illegible]
1005, 1013, 1109	Cultivation	300	28.1	20.9	16.7	13.6
1006, 1014, 1102, 1110	Sod	900	28.1	17.0	15.0	13.3
1007, 1015, 1103, 1111	Cultivation	900	28.3	20.9	17.0	14.0

*Detailed figures for this table are given in the appendix (page 25).

TABLE 3. Moisture Content in Surface Eight Inches of Soil in 1920*
(Averages for all plats receiving the same treatment)

Plats	Crop	Nitrate of soda applied (pounds per acre)	Moisture (per cent in dry soil)					
			April 13	May 18	June 11	June 29	July 15	Oct. [illegible]
1001, 1008, 1104, 1112	Sod	0	25.4	24.0	15.9	12.8	15.1	[illegible]
1009, 1105, 1113	Cultivation	0	25.4	22.3	19.0	16.7	16.9	20.[illegible]
1002, 1010, 1106, 1114	Sod	100	25.4	23.3	15.0	12.2	14.7	[illegible]
1003, 1011, 1107, 1115	Cultivation	100	24.4	21.9	19.3	17.4	17.6	19.5
1004, 1012, 1101, 1108	Sod	300	25.8	23.9	14.2	11.3	14.0	[illegible]
1005, 1013, 1109	Cultivation	300	24.8	22.6	19.3	17.4	18.0	[illegible]
1006, 1014, 1102, 1110	Sod	900	24.9	23.8	13.1	11.3	14.3	20.[illegible]
1007, 1015, 1103, 1111	Cultivation	900	24.6	22.2	19.8	18.0	18.3	18.[illegible]

*Detailed figures for this table are given in the appendix (page 26).

These figures show that in April there is little difference in the moisture content of the plats. Even by the middle of May the timothy

d not reduced the moisture below that of the cultivated soil. By ne, however, the effect of the grass was distinctly noticeable, and from at time until the rye was planted, the grass plats contained less moisre than did the cultivated ones. The analyses of October, on the other nd, show that the rye had by that time reduced the moisture lower an had the grass.

When the percentages of moisture in the plats receiving graduated antities of nitrate of soda are compared, it is seen that there was a ndency during June, when the grass was making its most rapid growth, r the moisture to be lower on the plats that received the larger applitions of the nitrate fertilizer. The moisture was low enough under the ass as compared with the cultivated plats to have a possible effect on e growth, but, as is shown later, the effect of the moisture was slight compared with that produced by the nitrates.

NITRATE NITROGEN IN THE SOIL

The quantity of nitrate nitrogen present in the surface eight inches soil on the same dates as those on which the moisture determinations re made, is recorded in tables 4 and 5:

TABLE 4. NITRATE NITROGEN IN SURFACE EIGHT INCHES OF SOIL IN 1919*
(Averages for all plats receiving the same treatment)

Plats	Crop	Nitrate of soda applied (pounds per acre)	Nitrate nitrogen (pounds per acre)			
			April 22	July 8	August 15	October 11
01, 1008, 1104, 1112	Sod	0	2.6	3.0	6.2	3.4
09, 1105, 1113	Cultivation	0	3.9	40.0	37.1	26.9
02, 1010, 1106, 1114	Sod	100	3.5	3.1	5.1	3.1
03, 1011, 1107, 1115	Cultivation	100	4.2	40.1	54.5	49.5
04, 1012, 1101, 1108	Sod	300	3.8	3.4	5.2	3.2
05, 1013, 1109	Cultivation	300	4.4	61.2	86.0	77.6
06, 1014, 1102, 1110	Sod	900	3.5	11.4	14.0	10.0
07, 1015, 1103, 1111	Cultivation	900	5.7	113.1	110.4	111.0

*Detailed figures for this table are given in the appendix (page 27).

TABLE 5. NITRATE NITROGEN IN SURFACE EIGHT INCHES OF SOIL IN 1920*
(Averages for all plats receiving the same treatment)

Plats	Crop	Nitrate of soda applied (pounds per acre)	Nitrate nitrogen (pounds per acre)					
			April 13	May 18	June 11	June 29	July 15	October 28
1, 1008, 1104, 1112	Sod	0	3.1	2.3	2.0	2.7	2.2	0
9, 1105, 1113	Cultivation	0	3.7	5.4	5.6	13.3	9.5	0
2, 1010, 1106, 1114	Sod	100	4.5	3.1	2.0	3.4	2.4	Trace
3, 1011, 1107, 1115	Cultivation	100	3.8	11.6	14.4	24.7	25.3	0
4, 1012, 1101, 1108	Sod	300	6.3	5.1	1.6	3.8	6.3	0
5, 1013, 1109	Cultivation	300	6.3	28.2	20.5	51.1	58.1	Trace
6, 1014, 1102, 1110	Sod	900	4.8	47.0	25.4	17.0	11.6	1.1
7, 1015, 1103, 1111	Cultivation	900	4.8	50.8	60.8	100.9	146.5	5.8

*Detailed figures for this table are given in the appendix (page 28).

It appears from tables 4 and 5 that between the dates when the rye was spaded under and when it was replanted in the summer, the nitrate nitrogen was much less in amount under timothy than in the cultivated soil. This difference increased as the season progressed. The differences are enormous, especially in the soil of the plats to which large quantities of nitrate of soda were applied. They are much greater than the differences in the moisture content of the corresponding plats. With the exception of the plats receiving the largest application of nitrate, scarcely any of the nitrate contained in the application of nitrate of soda remained in the soil of the timothy plats by the latter part of June. The nitrate nitrogen in the soil of the timothy plats steadily decreased in amount, while on the cultivated plats it increased.

DISAPPEARANCE OF NITRATE NITROGEN AFTER APPLICATION

It will be noticed that neither the cultivated plats nor the timothy plats which received heavy applications of nitrate of soda show enough nitrate nitrogen to account for that added in the form of the nitrate fertilizer. The late June and the July analyses disclose a larger proportional increase in the cultivated plats than in the timothy plats. For example, from the soil of the cultivated plats receiving 900 pounds of nitrate of soda, or 120 pounds of nitrate nitrogen, per acre, there was recovered in 1920 only about 45 pounds more nitrate nitrogen per acre on May 18 than from the plats that received no nitrate, 55 pounds more on June 11, 87 pounds more on June 29, and 137 pounds more on July 15. This suggests that the nitrate in the fertilizer had been converted into other forms of nitrogen, probably organic, soon after being applied to the soil, and later had been reconverted into nitrate. This hypothesis is supported by some data considered later.

RELATIVE IMPORTANCE OF MOISTURE AND NITRATE NITROGEN FOR TREE GROWTH

As was indicated at the beginning of this paper, one of the chief objects in undertaking the experiment was to ascertain whether the influence of sod on the soil moisture was the main cause for its injurious effect on tree growth on this soil, or whether its influence on the nitrate in the soil was the more potent factor. The experiment was not designed to throw any light on other possible causes, as, for example, the action of toxic substances. The experiment seems to furnish a very simple and obvious answer to the former question, so far, at least, as concerns apple trees on this particular soil. It will be noticed that in a number of cases the soil moisture content during the active growing season was low

on the sod plats receiving the largest applications of nitrate fertilizer. is is doubtless owing to the greater growth of timothy on these plats. wever, as is shown later, the tree growth on the sod plats was greatest ere the largest quantities of nitrate were applied, and consequently those plats where the soil moisture was often least during the growing ason. Since the tree growth was greatest where the soil moisture was st, it is evident that the relatively low moisture under grass was not a ry important factor in curtailing the growth. On the other hand, the t that tree growth on the sod plats was greatest where the greatest antities of nitrate of soda were applied, is evidence that nitrate nitrogen s an important consideration.

OTHER POSSIBLE EFFECTS OF CULTIVATION ON TREE GROWTH

While the importance of moisture is thus minimized, it is perhaps t safe to attribute to the nitrates sole credit for the benefit that the ees obtained from cultivation of the soil. Glancing again at tables 4 d 5, it will be seen that in the soil of the cultivated plats the nitrate trogen was greatest during the growing season where the nitrate rtilizer applications were the greatest. On the other hand, it is dissed by tables 8 and 9 that tree growth was not greatest in the cultvated plats in which nitrate nitrogen was the highest. This may have en because all of the cultivated plats, whether thus fertilized or not, ntained an adequate supply or even a surplus of nitrate nitrogen. If at were the case, the addition of nitrate fertilizer could not be expected produce differences in tree growth.

While this explanation will probably account for the major part of benefit which the trees derived from cultivation, there remains the sibility that this treatment brought about some condition, other than duction of nitrates, that benefited tree growth. As a further indication his, it will be observed that the sod plats receiving 900 pounds of ate of soda per acre contained a higher amount of nitrate nitrogen out the spring and summer than did the cultivated plats receiving nitrogen, and the former plats were higher in nitrate nitrogen content ing May and the first half of June than were the cultivated plats eiving 100 and 300 pounds of nitrate of soda, respectively. In spite this high content of nitrate nitrogen in the soil of the sod plats to ich 900 pounds of nitrate of soda had been applied, the tree growth these plats was only about two-thirds as great as on the cultivated ts that received no nitrogen.

ERVATION OF NITRATE NITROGEN BY ITS CONVERSION INTO OTHER FORMS

Attention has been called to the disappearance of nitrates in the of the sod plats. In 1919 an application of 120 pounds of nitrate

nitrogen per acre had been reduced to 11.4 pounds by July 8, and in 1920 a similar amount had been reduced to 11.6 pounds by July 15. These analyses were made immediately following the cutting of the hay, which was on July 7 and July 12, respectively. If no nitrates were formed from the soil supply of nitrogenous substances during the growth of the grass, there would have been approximately 108 pounds of nitrate nitrogen that had disappeared each year. But it will be remembered that the plats receiving no nitrate fertilizer produced a crop of timothy about half as large as the other. It would therefore be untenable to assume that no nitrates had been formed during the growth of the crop, but this source of nitrogen is purposely ignored in the calculations in table 6, showing the disappearance of nitrate nitrogen.

TABLE 6. DISAPPEARANCE OF APPLIED NITRATE NITROGEN FROM SOD PLATS IN 1921

Plats	Nitrate of soda applied (pounds per acre)	Nitrate nitrogen (pounds per acre)						
		Present in soil April 25	Applied to soil April 25	Total in soil April 25	Present in soil July 12	Gain (+) or loss (–) between April 25 and July 12	Contained in hay crops July 12	Loss not due to removal by crops
1008, 1104, 1112	0	0.51	0	0.51	2.77	+2.26	18.12	—
1010, 1106, 1114	100	1.24	13.33	14.57	2.37	–12.20	20.91	—
1004, 1012, 1108	300	0.68	39.99	40.67	3.33	–37.34	34.59	2.75
1006, 1014, 1110	900	5.42	119.97	125.39	10.11	–115.28	70.14	45.14

The quantity of nitrogen absorbed by the trees could not be determined easily, but that contained in the hay crop could be; consequently it was decided not to try to ascertain what became of the nitrate nitrogen until the trees should have been removed. In 1921, the trees being no longer on the land, the timothy plats were fertilized as usual and the grass was allowed to grow. Samples of soil for determination of nitrates were taken immediately before applying the fertilizer on April 25 and immediately after cutting the hay on July 12. The hay was weighed, samples were taken, and the total nitrogen was determined.

In table 6 may be found a statement of the amounts of nitrate nitrogen present in the soil before applying fertilizer in the spring and after removing the hay, and of the amounts applied to the soil. From these data are calculated the gains or losses of nitrogen from the soil between April 25, when the fertilizer was applied, and July 12, which was shortly after the hay crops had been removed. Figures are given also showing the amounts of nitrogen in the hay crops, and the last column of the table shows the losses of nitrate nitrogen that could not be accounted for by absorption by the growing timothy. Hay on the plats receiving

nitrate of soda contained 18.12 pounds of nitrogen per acre. Pre-mably this nitrogen was mainly in the form of nitrates before it was sorbed by the plants. Where nitrate of soda was applied, nitrification ay have been less, and consequently this supply of nitrate nitrogen is not considered in computing the losses not due to removal by crops, hich therefore may possibly have amounted to 18 pounds more than shown in the table.

As is indicated in table 6, in the sod plats receiving the larger quan-ties of nitrate there was a very considerable disappearance of nitrogen om the nitrate condition. A part of the nitrogen unaccounted for was ntained in the roots and the stubble of the timothy sod, but this is as e of the unfertilized plats as of the fertilized. It is therefore not that th which the present study is concerned. Removal in the drainage ter would probably not account for much of the disappearance, if the ainage from the lysimeter tanks is taken as the criterion. Grass has en grown for nine years consecutively on certain tanks, and during at time the average annual removal of nitrate nitrogen in the drainage ter was 1.45 pounds, while the largest removal in any one year was 8 pounds. The same type of soil was used in the lysimeter as in this periment.

There is evidently a very considerable quantity of the nitrate ogen that cannot be accounted for by (1) removal in the hay crop, incorporation in the roots and stubble, or (3) removal in the drain-water. As has already been mentioned, the growth of timothy, and less extent of the cereals also, usually reduces the nitrates in the soil a low figure. Apparently, part of this transformation is due to the sumption of the nitrate nitrogen by soil organisms whose growth is ored by the sod, with the result that the nitrate nitrogen is converted o other compounds, in which form it may be held for some time, and en conditions are favorable for the nitrifying process, it may again be verted into nitrate.

The growth of rye on the cultivated plats in 1921 also gives an indi-n of how these transformations take place. In that year, no nitrate la was applied to any of the cultivated plats, but the rye was allowed ow to maturity instead of being plowed under in the spring, as had ously been done. It is significant that the relative growth of rye ese plats was in the same order as the previous applications of nitrate la, altho the effect of these treatments on the nitrate content of the ad largely disappeared by the autumn of 1920. In table 7 may be l a statement of the quantities of nitrate of soda applied annually, itrate content of the several plats in the autumn of 1920, and the s of rye, both grain and straw, in 1921:

TABLE 7. Yields of Rye on Plats Previously Treated with Different Quantities of Nitrate of Soda

Plats	Nitrate of soda applied annually previous to 1921 (pounds per acre)	Nitrates October 28, 1920 (parts per million)	Yield of rye in 1921 (pounds per acre)
1009, 1105, 1113	0	0	900
1003, 1011, 1107, 1115	100	0	1,250
1005, 1013, 1109	300	Trace	1,800
1007, 1015, 1103, 1111	900	10.2	3,787

It would appear that the nitrate of soda had not been entire leached from the soil, but that, while nitrates had almost complete disappeared in the fall of 1920, there still remained a part of the nitrog that had been transformed by soil organisms into other forms. It true that somewhat more nitrogen had been turned under in the r cover-crops on the plats receiving large quantities of nitrate of soda, b in no case did the rye grow very tall, as it was plowed under early the spring; so that the differences in this respect were not very grea It does not seem likely, therefore, that differences in the amounts of nitr gen plowed under in the rye could have accounted for the large diffe ences in the yields of rye on these plats. Microorganisms probably h more to do with the conversion of nitrate nitrogen into organic matt in the soil than did absorption by the rye.

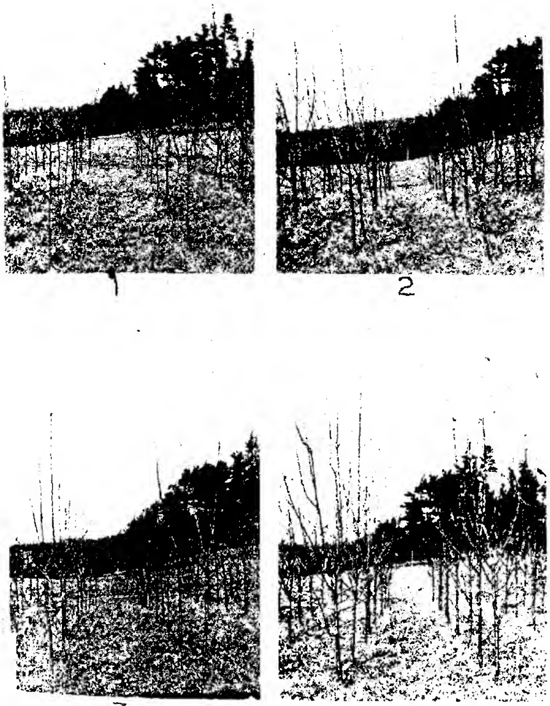
RESPONSE OF TREES TO SOIL TREATMENTS

The differences in size and vigor on corresponding sod and cul vated plats receiving varying amounts of nitrate of soda are indicat in Plates I, II, and III.

Weight of trees

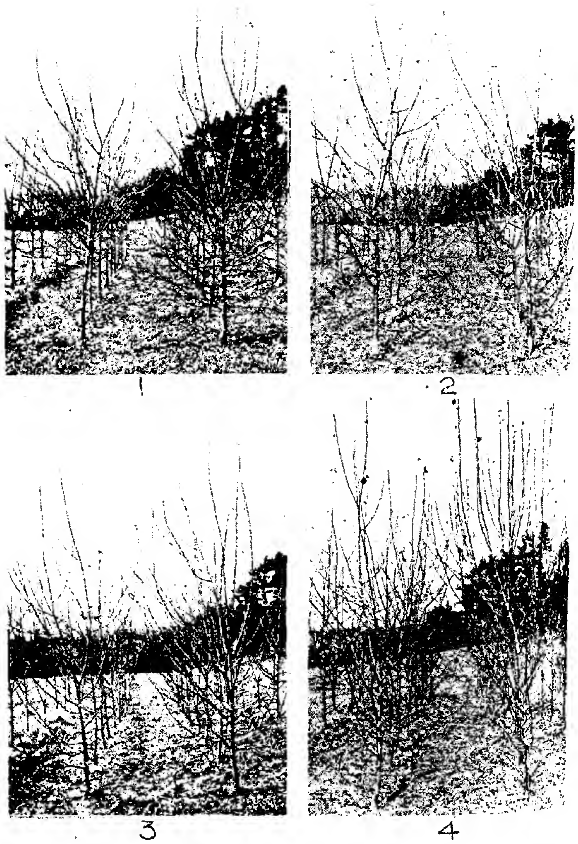
The data concerning the average weight of trees from the differe plats are given in table 8. The probable error for the average of t eighteen to twenty healthy trees of each plat was calculated by the form

$$E_{\text{mean}} = \pm 0.6745 \sqrt{\frac{\Sigma D^2}{n^2}}$$



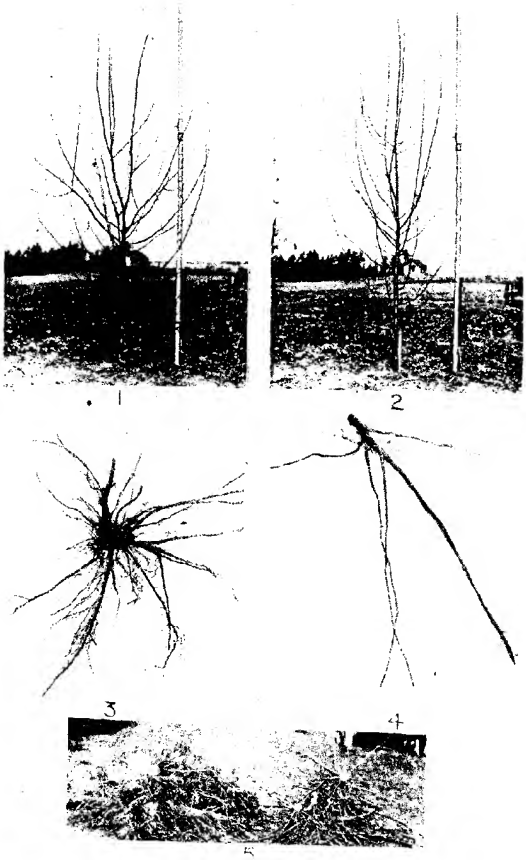
EFFECT OF SODIUM NITRATE ON TREES IN SOD

1, Plat 1008, no sodium nitrate applied
2, Plat 1010, application 100 pounds
3, Plat 1012, application 300 pounds
4, Plat 1006, application 900 pounds



EFFECT OF SODIUM NITRATE ON TREES ON CULTIVATED PLATS

1, Plat 1009, no sodium nitrate applied
2, Plat 1011, application 100 pounds
3, Plat 1013, application 300 pounds
4, Plat 1007, application 900 pounds



EFFECT OF CULTIVATION ON TREE AND ROOT GROWTH

1, Vigorous tree from cultivated plat, showing many strong branches. 2, Vigorous tree from sod, showing characteristic long terminal growth with few branches. 3, Typical root system from cultivated tree. 4, Typical root system from tree in sod. 5, Fibrous roots from cultivated trees, and non-fibrous roots from sod trees.

TABLE 8. EFFECT OF SOIL TREATMENTS ON WEIGHT OF TREES

Nitrate of soda applied (pounds)		Timothy sod		Cultivation, rye cover-crop		Increase of cultivation over sod (grams)	Average percentage difference between trees of same rank in cultivated and adjoining sod plats
Per acre	Per tree	Plat	Average weight of trees (grams)	Plat	Average weight of trees (grams)		
0	0	1001	1,425				
		1008	1,120 ± 120	1009	4,660 ± 260	3,540 ± 286	439 ± 23.2
		1104	1,420 ± 93	1105	4,790 ± 247	3,370 ± 264	274 ± 12.8
		1112	1,650 ± 100	1113	4,660 ± 175	3,010 ± 201	247 ± 24.3
		Average	1,404 ± 73		4,703 ± 30	3,307 ± 86	
100	1/20	1002	980 ± 100	1003	4,460 ± 280	3,480 ± 297	375 ± 16.2
		1010	1,310 ± 67	1011	4,775 ± 362	3,465 ± 368	240 ± 11.9
		1106	1,630 ± 85	1107	4,800 ± 337	3,170 ± 346	200 ± 6.8
		1114	1,390 ± 118	1115	5,410 ± 351	4,020 ± 370	353 ± 11.1
		Average	1,328 ± 91		4,861 ± 134	3,534 ± 162	
300	3/20	1004	1,110 ± 93	1005	5,270 ± 316	4,160 ± 330	439 ± 15.5
		1012	1,985 ± 121	1013	4,630 ± 285	2,645 ± 310	138 ± 5.0
		1101	1,483				
		1108	1,830 ± 94	1109	5,170 ± 341	3,340 ± 352	187 ± 5.5
		Average	1,602 ± 130		5,023 ± 132	3,382 ± 185	
900	9/20	1006	2,905 ± 131	1007	5,170 ± 305	2,265 ± 332	68 ± 4.6
		1014	2,065 ± 191	1015	5,150 ± 304	3,085 ± 359	193 ± 12.2
		1102	3,695 ± 221	1103	5,005 ± 320	1,310 ± 390	27 ± 2.5
		1110	3,550 ± 267	1111	4,730 ± 305	1,180 ± 412	43 ± 4.1
		Average	3,054 ± 251		5,014 ± 78	1,960 ± 264	

and that for the average of the three or four plats receiving the same treatment, by the formula

$$E_{mean} = \pm\ 0.6745 \sqrt{\frac{\Sigma D^2}{n(n-1)}}$$

It is plainly evident that the growth of the trees was retarded by sod as compared with cultivation. The difference between the average for any sod plat and the average for the adjoining cultivated plat with the same application of nitrate of soda, was greater than three times the

probable error in all cases except for plats 1110 and 1111. When the averages of all plats receiving the same fertilizer treatments are considered, the difference between sod and cultivation is many times the probable error. The average percentage of difference between the trees of the same rank (that is, the largest, the next largest, and so forth, in a given cultivated plat as compared with the trees of corresponding rank in an adjoining sod plat with the same fertilizer treatment) indicates even more strikingly the harmful influence of the sod.

The application of nitrate of soda at the rate of 900 pounds per acre, which amounted to a little less than ½ pound per tree, had a marked influence in reducing the injurious effect of timothy sod. The trees from the sod plats receiving this application averaged more than twice the weight of the trees from sod plats without the addition of nitrate of soda. Nevertheless, such an application was not sufficient to entirely overcome the influence of sod, as is indicated by the fact that the trees receiving this treatment weighed less than two-thirds as much as those from cultivated plats without nitrate fertilization. An application of 100 pounds of nitrate of soda per acre apparently had no influence in reducing the injury from sod, but some effect was evident from the use of 300 pounds per acre.

In cultivated plats the addition of nitrate nitrogen resulted in little, if any, increase in the weight of the trees. When any two near-by plats, such as 1009 and 1007, are compared, the difference is too small to be significant when calculated by the formula

$$E_{\text{diff.}} = \sqrt{E_1^2 + E_2^2}$$

There certainly is no significant difference among the cultivated plats receiving varying amounts of nitrate of soda; for example, an application of 300 pounds per acre proved just as effective as one of 900 pounds. The cover crop of rye, on the other hand, as previously noted, showed a marked response to the application of nitrates.

It should be noted that the relatively small area used for these experiments afforded much more uniform soil conditions than would obtain in a larger area. Nevertheless, in several cases, groups of trees receiving the same treatment showed marked differences in growth; for example, plats 1014 and 1102, or plats 1004 and 1012. Such differences in response are not entirely accounted for by variations in nitrates and moisture on the dates when these soil factors were determined, as is shown by the detailed tables 2, 3, 4, and 5 in the appendix. It is evident, from a study of the data, that the trees responded to other conditions in addition to those revealed by the nitrate and moisture determinations.

Circumference of trees

The data concerning the average circumference of the tree trunks (table 9), altho not so reliable as those for the final weight, indicate the response of the trees from year to year. During the first season,

TABLE 9. Effect of Soil Treatments on Girth of Trees

Nitrate of soda applied (pounds per acre)	Average circumference for given years (in centimeters)									
	Timothy sod					Cultivation, rye cover-crop				
	Plat	1917	1918	1919	1920	Plat	1917	1918	1919	1920
0	1001	4.1	5.0	6.1	8.4					
	1008	4.1	5.1	5.8	7.8	1009	4.1	6.9	9.6	13.0
	1104	4.1	4.8	5.8	8.6	1105	4.1	6.8	9.8	13.2
	1112	4.0	4.8	6.3	9.2	1113	4.1	6.8	9.7	13.3
Average		4.1	4.9	6.0	8.5		4.1	6.8	9.7	13.2
100	1002	4.0	4.7	5.5	7.3	1003	4.1	6.8	9.4	13.2
	1010	4.0	5.1	6.3	8.6	1011	4.0	6.5	9.4	13.0
	1106	4.3	5.1	6.4	9.2	1107	4.1	6.6	9.4	13.0
	1114	4.1	4.7	6.2	8.4	1115	4.1	6.5	9.6	13.5
Average		4.1	4.9	6.1	8.4		4.1	6.6	9.5	13.3
300	1004	4.0	4.9	6.0	7.9	1005	4.1	7.3	10.1	13.8
	1012	4.1	5.2	6.8	9.8	1013	4.1	6.9	9.5	13.2
	1101	4.0	4.6	6.1	8.8					
	1108	4.1	5.1	6.7	9.7	1109	4.2	6.6	9.6	13.2
Average		4.1	5.0	6.4	9.1		4.1	6.9	9.7	13.4
900	1006	4.1	6.1	8.2	11.2	1007	4.1	7.0	9.8	13.4
	1014	4.0	5.2	6.9	9.8	1015	4.1	6.9	9.7	13.0
	1102	4.1	5.9	8.4	12.0	1103	4.1	6.7	9.2	12.9
	1110	4.1	5.7	8.1	11.7	1111	4.2	6.6	9.4	13.0
Average		4.1	5.7	7.9	11.2		4.1	6.8	9.5	13.3

when all plats received the same treatment, the original average uniformity of size as indicated by girth was maintained. Apparently the soil variations which became manifest in subsequent years had but little effect on the tree growth during this first season. The trees immediately responded to the various treatments in 1918, and the lead established that year was maintained during the course of the experiment.

Height and spread of trees

The measurements of the height and spread of the trees, given in able 10, also corroborate the results previously discussed. It is inter-sting to note that the average height of the trees grown on the timothy lats receiving 900 pounds of nitrate of soda per acre is as great as that

TABLE 10. EFFECT OF SOIL TREATMENTS ON HEIGHT AND SPREAD OF TREES

Nitrate of soda applied (pounds per acre)	Timothy sod				Cultivation, rye cover-crop			
	Plat	Average spread (feet)	Average height (feet)	Average ratio	Plat	Average spread (feet)	Average height (feet)	Average ratio
0	1008	2.5	5.5	2.20	1009	4.1	8.1	1.98
	1104	2.8	6.3	2.25	1105	4.1	8.7	2.12
	1112	3.2	6.5	2.03	1113	4.4	8.2	1.86
Average		2.8	6.1	2.18		4.2	8.3	1.98
100					1003	4.3	7.9	1.84
	1010	2.8	6.4	2.29	1011	3.9	8.2	2.10
	1106	3.0	6.4	2.13	1107	4.2	8.3	1.98
	1114	3.0	5.6	1.87	1115	4.6	8.3	1.80
Average		2.9	6.1	2.10		4.3	8.2	1.91
300	1004	2.5	6.1	2.44	1005	4.3	9.1	2.12
	1012	2.9	7.0	2.41	1013	4.1	8.1	1.98
	1108	2.9	7.2	2.48	1109	4.4	8.6	1.95
Average		2.8	6.8	2.43		4.3	8.6	2.00
900	1006	2.9	8.2	2.83	1007	4.2	8.3	1.98
	1014	2.6	7.3	2.81	1015	4.5	8.2	1.82
	1102	3.2	8.3	2.59	1103	4.1	8.2	2.00
	1110	3.6	8.8	2.44	1111	4.5	8.1	1.80
Average		3.1	8.2	2.65		4.3	8.2	1.91

the trees on the cultivated plats, but the spread is less. The trees sod plats receiving 300 pounds of nitrate of soda per acre are some-at higher than those receiving less fertilizer. Relatively few strong inches are formed on the more vigorous trees grown on sod, and a

large proportion of the lateral buds remain dormant, as compared with trees on cultivated plats. The stimulation resulting from the application of nitrates in the case of trees in sod seems to be manifest primarily in additional length growth. This is shown clearly in Plate I.

Effect of soil conditions on the root system

The ratio between the weight of the top and the weight of the root system may be affected by treatments or conditions that favor rapid and prolonged top growth (Chandler, 1919). Apparently the soil treatment, as well as the location of the respective plats on the experimental area, influenced this relationship, as indicated by the data in table 11:

TABLE 11. RATIO BETWEEN WEIGHT OF TOP AND WEIGHT OF ROOT SYSTEM

Nitrate of soda applied (pounds)		Timothy sod				Cultivation, rye cover-crop			
			Average weight				Average weight		
Per acre	Per tree	Plat	Roots (grams)	Tops (grams)	Average ratio	Plat	Roots (grams)	Tops (grams)	Average ratio
0	0	1008	385	735	1.91	1009	1,500	3,160	2.11
		1104	509	911	1.79	1105	1,610	3,180	1.98
		1112	650	1,000	1.54	1113	1,730	2,930	1.69
		Average	515	882	1.71		1,613	3,090	1.92
100	1/20	1010	436	874	2.00	1011	1,585	3,190	2.01
		1106	565	1,065	1.88	1107	1,520	3,280	2.16
		1114	491	899	1.83	1115	2,110	3,300	1.56
		Average	497	946	1.90		1,738	3,257	1.87
300	3/20	1004	389	721	1.85	1005	1,750	3,520	2.01
		1012	640	1,345	2.10	1013	1,575	3,055	1.94
		1108	615	1,215	1.98	1109	1,765	3,405	1.93
		Average	548	1,094	2.00		1,697	3,327	1.96
900	9/20	1006	945	1,960	2.07	1007	1,840	3,330	1.81
		1014	619	1,446	2.34	1015	1,645	3,505	2.13
		1102	1,170	2,525	2.16	1103	1,665	3,340	2.01
		1110	1,090	2,460	2.26	1111	1,700	3,030	1.78
		Average	956	2,098	2.19		1,713	3,301	1.93

The trees on the sod plats receiving no nitrate nitrogen had relatively heavy roots for the small tops, as compared with the corresponding cultivated plats. However, the roots from trees in the sod plats receiving 900 pounds of nitrate of soda to the acre, constituted a much smaller part of the total weight of the tree. The average ratio for the trees on sod plats with 100 pounds and with 300 pounds of nitrate of soda fell tween these extremes.

In the case of trees in the cultivated plats, the application of nitrate trogen apparently did not affect the relationship between weight of ie top and weight of the root system. The roots on all trees from such ats were distinctly fibrous, as compared with the long, sparsely branched oots of the trees on sod, as indicated in Plates II and III. This was the ise irrespective of the fertilizer treatment.

SUMMARY

Apple trees were grown on field plats continuously in sod, and on lats on which rye was used as a cover crop. All plats were fertilized ith acid phosphate and muriate of potash. Nitrate of soda was applied o certain of the sod and cover-crop plats at the respective rates of 900, 00, and 100 pounds per acre, and was withheld entirely from others.

Moisture and nitrates in the soil were determined from time to me. Measurements were made of the tree growth at the end of each ason. At the end of the experiment, the trees were cut off at the surface the soil and weighed, and the roots were dug and weighed.

Moderate differences in moisture content of the soil were observable etween the variously treated plats, but they were slight as compared ith the differences in the nitrate nitrogen present. Nitrates were always w under the sod except when large quantities of nitrate of soda had en applied recently.

Tree growth was greatest on those sod plats which received the atest quantity of nitrate of soda, indicating a deficiency of available rogen under the unfertilized sod.

That the removal of moisture from the soil by the grass was not an portant factor in tree growth was indicated by the fact that the growth the trees was greatest on those sod plats in which the moisture was st, owing to a greater growth of grass resulting from the large applicams of nitrate of soda. Apparently the maintenance of an adequate pply of nitrate nitrogen in the soil used in this experiment was the termining factor in tree growth, and soil moisture was very much less portant.

There was a disappearance of nitrate nitrogen from the soil of the plats which could not be accounted for by its removal in the crops

of hay or its incorporation in the roots and stubble, and presumably not by its removal in drainage water. It is probable that the consumption of nitrate nitrogen by soil organisms caused the disappearance by converting the nitrate nitrogen into other compounds.

Even on the cover-crop plats this transformation of nitrogen apparently occurred, as is indicated by the growth of rye on the plats in 1921 when, altho no nitrate was applied that year, the yield was in the same order as the quantity of nitrate of soda applied in previous years. Practically all nitrate nitrogen had disappeared from the soil of all the plats in the autumn of 1920, so that the larger quantity of available nitrogen on the nitrate-of-soda plats must have come from the nitrification of nitrogenous material, most likely in organic combination, that had previously been transformed from nitrate.

The injurious effect of sod on the growth of young apple trees was reduced by the annual application of ½ pound of nitrate of soda per tree.

Trees on cultivated plats did not respond to the addition of nitra fertilizer, whereas those on sod plats receiving ½ pound per tree ave aged more than twice the weight of those on sod plats without nitra

Trees on sod plats receiving nitrate of soda showed vigorous termin growth, but relatively few strong branches as compared with trees o cultivated plats.

Trees on sod plats receiving no nitrate nitrogen had relatively heavy roots as compared with those on cultivated plats, but the roots from trees on sod plats receiving heavy applications of nitrate constituted a much smaller part of the total weight of the tree.

The roots from trees on cultivated plats were more fibrous as compared with those from trees on sod plats.

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APPENDIX

BLE 2 (IN DETAIL). MOISTURE CONTENT IN SURFACE EIGHT INCHES OF SOIL IN 1919

itrate f soda pplied pounds r acre)	Timothy sod Moisture (per cent in dry soil)					Cultivation Moisture (per cent in dry soil)				
	Plat	April 22	July 8	Aug. 15	Oct. 11	Plat	April 22	July 8	Aug. 15	Oct. 11
0	1001	26.1	15.6	14.8	16.4					
	1008	26.8	16.2	17.2	18.6	1009	26.5	19.9	15.8	12.6
	1104	28.5	18.0	19.7	20.3	1105	26.7	19.6	16.1	11.3
	1112	30.0	18.6	19.4	19.6	1113	29.5	20.1	16.2	12.8
Average		27.8	17.1	17.8	18.7		27.6	19.9	16.0	12.2
100	1002	27.5	15.8	17.2	18.0	1003	27.5	19.7	16.0	12.7
	1010	28.2	16.8	18.6	18.7	1011	27.3	20.4	16.9	14.1
	1106	26.8	16.6	18.7	19.3	1107	26.7	20.4	17.3	13.1
	1114	30.5	18.0	19.1	20.0	1115	28.5	21.3	16.9	13.1
Average		28.2	16.8	18.4	19.0		27.5	20.4	16.8	13.2
300	1004	28.5	16.4	16.2	16.8	1005	27.7	20.6	16.5	13.5
	1012	28.5	17.0	17.7	16.8	1013	28.5	20.9	17.0	14.4
	1101	27.2	16.8	18.9	19.0					
	1108	28.5	16.9	19.3	18.3	1109	28.0	21.2	16.5	12.8
Average		28.2	16.8	18.0	17.7		28.1	20.9	16.7	13.6
00	1006	27.0	16.2	14.0	12.3	1007	26.5	20.1	16.4	13.0
	1014	29.5	16.9	13.3	12.3	1015	31.0	20.6	16.6	13.3
	1102	27.5	16.6	15.2	13.5	1103	27.5	20.0	16.9	14.0
	1110	28.5	18.3	17.7	15.3	1111	28.3	23.1	18.2	15.7
Average		28.1	17.0	15.0	13.3		28.3	20.9	17.0	14.0

TABLE 3 (IN DETAIL). MOISTURE CONTENT IN SURFACE EIGHT INCHES OF SOIL IN 1920

Nitrate of soda applied (pounds per acre)	Timothy sod Moisture (per cent in dry soil)							Cultivation Moisture (per cent in dry soil)						
	Plat	April 13	May 18	June 11	June 29	July 15	Oct. 28	Plat	April 13	May 18	June 11	June 29	July 15	Oct. 28
0	1001	23.3	22.2	13.6	9.6	13.2	22.2							
	1008	25.0	23.3	15.2	12.8	15.0	25.1	1009	24.5	21.3	18.7	16.9	18.6	22.5
	1104	26.2	24.5	17.6	14.4	16.1	25.3	1105	24.3	21.6	18.3	16.2	16.0	19.0
	1112	27.3	26.2	17.5	14.5	16.2	26.1	1113	27.3	24.0	20.0	17.0	16.1	19.0
	Average	25.4	24.0	15.9	12.8	15.1	24.7		25.4	22.3	19.0	16.7	16.9	20.2
100	1002	23.7	21.9	13.6	10.0	14.4	23.0	1003	24.3	21.6	19.1	17.6	17.7	20.0
	1010	25.4	22.8	15.3	12.8	15.2	25.0	1011	24.6	21.6	19.3	17.6	18.2	21.6
	1106	25.3	23.0	15.2	12.6	14.7	24.3	1107	23.6	21.5	19.0	16.8	16.9	19.7
	1114	27.3	25.6	16.0	13.3	14.7	26.2	1115	25.3	22.8	19.7	17.5	17.5	16.8
	Average	25.4	23.3	15.0	12.2	14.7	24.6		24.4	21.9	19.3	17.4	17.6	19.5
300	1004	26.1	23.0	12.1	11.3	14.0	25.0	1005	24.2	21.9	19.1	17.7	18.2	19.9
	1012	26.5	24.3	15.2	11.8	15.0	25.4	1013	25.3	23.6	19.7	17.6	17.9	19.0
	1101	25.3	24.0	14.2	10.1	12.7	23.4							
	1108	25.4	24.3	15.4	11.9	14.4	24.0	1109	25.0	22.4	19.1	16.9	17.9	17.9
	Average	25.8	23.9	14.2	11.3	14.0	24.4		24.8	22.6	19.3	17.4	18.0	18.9
900	1006	24.3	23.3	12.3	9.4	13.0	21.3	1007	23.6	21.3	18.3	17.7	16.9	19.0
	1014	26.5	25.1	13.3	10.8	14.2	22.1	1015	25.7	23.1	20.4	18.9	18.9	16.9
	1102	24.8	22.5	13.7	10.9	14.0	18.9	1103	24.2	22.1	19.1	16.6	18.3	18.0
	1110	24.2	24.2	13.2	14.0	16.2	20.6	1111	25.0	22.5	21.3	18.9	19.3	18.6
	Average	24.9	23.8	13.1	11.3	14.3	20.7		24.6	22.2	19.8	18.0	18.3	18.1

TABLE 4 (IN DETAIL). NITRATES IN SURFACE EIGHT INCHES OF SOIL IN 1919

Nitrate of soda applied (pounds per acre)	Timothy sod Nitrates (NO_3) (parts per million in dry soil)					Cultivation Nitrates (NO_3) (parts per million in dry soil)				
	Plat	April 22	July 8	Aug. 15	Oct. 11	Plat	April 22	July 8	Aug. 15	Oct. 11
0	1001	7.9	7.1	21.2	4.0					
	1008	4.0	4.8	6.9	4.9	1009	7.2	74.0	55.1	51.7
	1104	2.2	4.9	8.9	9.3	1105	9.2	79.0	81.2	45.2
	1112	4.5	4.3	6.8	5.7	1113	4.5	59.7	60.9	46.2
Average		4.7	5.3	10.9	6.0		7.0	70.9	65.7	47.7
100	1002	8.0	7.1	7.5	4.9	1003	8.0	64.2	119.4	103.9
	1010	6.7	6.0	7.9	4.9	1011	10.0	87.1	115.0	84.9
	1106	5.9	5.1	11.1	7.4	1107	3.5	62.2	72.6	92.3
	1114	4.5	3.7	9.8	4.9	1115	8.0	70.2	79.2	69.2
Average		6.3	5.5	9.1	5.5		7.4	70.9	96.6	87.6
300	1004	13.4	6.6	8.1	6.6	1005	7.3	104.5	143.3	116.0
	1012	5.4	6.0	12.1	4.8	1013	8.0	100.3	163.2	146.3
	1101	4.4	6.0	6.8	4.5					
	1108	3.6	5.4	9.8	6.5	1109	8.0	120.4	150.4	150.0
Average		6.7	6.0	9.2	5.6		7.8	108.4	152.3	137.4
900	1006	4.4	28.7	21.1	11.5	1007	9.9	199.0	200.1	198.6
	1014	7.5	16.8	15.1	6.1	1015	11.0	174.2	252.0	150.8
	1102	7.3	19.2	14.8	15.1	1103	12.0	197.4	115.8	198.9
	1110	5.4	15.9	48.4	38.0	1111	7.4	230.4	213.5	237.2
Average		6.2	20.2	24.9	17.7		10.1	200.3	195.4	196.4

TABLE 5 (IN DETAIL). NITRATES IN SURFACE EIGHT INCHES OF SOIL IN 1920

Nitrate of soda applied (pounds per acre	Timothy soil Nitrates (NO_3) (parts per million in dry soil)							Cultivation Nitrates (NO_3) (parts per million in dry soil)						
	Plat	April 13	May 18	June 11	June 29	July 15	Oct. 28	Plat	April 13	May 18	June 11	June 29	July 15	Oct. 28
0	1001	Trace	3.4	4.6	Trace	2.8	None							
	1008	9.1	5.1	4.7	6.9	5.3	None	1009	7.7	9.4	12.3	24.0	21.9	None
	1104	7.9	4.3	4.8	6.4	4.2	None	1105	6.5	12.6	9.8	29.9	19.1	None
	1112	5.3	3.5	Trace	5.9	3.0	None	1113	5.3	6.8	7.4	16.8	9.6	None
Average		5.6	4.1	3.5	4.8	3.8	None		6.5	9.6	9.8	23.6	16.9	None
100	1002	11.0	4.6	5.2	6.7	4.1	Trace	1003	7.7	27.7	43.0	51.4	60.5	None
	1010	7.9	4.2	Trace	6.9	4.1	None	1011	6.5	20.2	22.1	54.5	51.2	None
	1106	7.8	10.2	8.9	6.3	5.9	None	1107	7.7	13.8	22.1	36.0	31.2	None
	1114	5.3	3.1	Trace	4.1	2.9	None	1115	5.2	20.3	14.8	33.3	36.3	None
Average		8.0	5.5	3.5	6.0	4.2	None		6.8	20.5	25.5	43.8	44.8	None
300	1004	15.7	13.3	5.7	7.9	3.5	None	1005	14.2	60.5	52.2	109.0	107.4	Trace
	1012	11.9	10.3	5.9	6.9	35.4	None	1013	7.8	33.3	32.1	69.6	97.6	Trace
	1101	6.5	7.7	Trace	5.6	2.9	None							
	1108	10.5	5.2	Trace	6.8	2.9	None	1109	11.7	55.9	24.6	93.0	103.7	None
Average		11.2	9.1	2.9	6.8	11.2	None		11.2	49.9	36.3	90.5	77.2	None
900	1006	11.6	97.3	57.4	20.0	13.8	4.2	1007	7.7	70.2	112.9	157.3	312.0	6.7
	1014	6.6	93.6	27.8	9.1	31.6	Trace	1015	9.2	102.4	102.6	196.5	302.7	6.0
	1102	9.1	91.4	58.0	38.4	17.4	None	1103	11.6	131.0	135.1	198.0	244.0	17.1
	1110	6.5	56.8	36.9	52.7	19.7	3.3	1111	5.2	55.9	80.0	162.7	196.8	11.0
Average		8.4	84.8	45.0	30.1	20.6	1.9		8.4	89.9	107.6	178.6	263.9	10.2

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ATURE AND REACTION OF WATER FROM HYDATHODES

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ITHACA, NEW YORK
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THE NATURE AND REACTION OF WATER FROM HYDATHODES

J. K. WILSON

In the process of growth and development, plants lose various parts f their structure. Root hairs are, relatively speaking, of short duration, nd root-cap cells are gradually sloughed off; while pollen and other floral arts are soon lost. In addition to this loss of organic material, the lants return to the soil, by a gradual passing downward and outward hrough the root system, various inorganic and organic materials. These aterials may be lost also through special organs, such as the nectaries r the hydathodes, the materials either falling off or being washed away y rain or dew.

In studying the effect that plants have on the growth of bacteria in oil, it became desirable, in order to throw light on the results that were eing obtained, to make a study of the presence of certain materials in e exudate water of maize, oats, and timothy. This paper gives the sults of the findings from this study, in so far as they bear on the broader vestigation.

PREVIOUS STUDIES

An investigation somewhat similar to this was pursued by Berthelot d reported by Duchartre (1859). Four hundred cubic centimeters guttation water was collected from Colocasia and evaporated to dry-ss. The residue contained potassium chloride, calcium carbonate, d a mucilaginous material. The last-named was completely soluble all concentrations and produced a froth when boiled. When the dry sidue was heated, it carbonized. It is concluded, however, that only e merest traces of organic and inorganic materials were found in this udate, and that the concentration was about equal to that of distilled ater.

Marloth (1887), in Egypt, collected the salts from the leaves and stems Tamarix. The dry salts consisted of $CaCO_3$ 51.9 per cent, $MgSO_4H_2O$ per cent, $MgCl_2$ 4.7 per cent, $MgHPO_4$ 3.2 per cent, NaCl 5.5 per cent, aNO_3 17.2 per cent, and Na_2CO_3 3.8 per cent.

Lepeschkin (1906) analyzed water from the secreting cells of a number plants. In addition to a considerable number of inorganic substances, rtain organic compounds were found. Glucose was secreted from *cia sativa* and *Polypodium aureum*, while basic oxalic acid was found the water from *Lathyrus odoratus.*

The forms of nitrogen in plants were studied by Klein (1913). Guttation ter was collected and examined, diphenylamine and nitron being d as reagents. Klein concluded that nitrates were not found in the date water from *Splitgerbera biloba*, *Fuchsia* sp., *Nicotiana silvestris*, *adescantia viridis*, *Tolmiaea Menziesii*, and *Zea Mays* seven weeks old, t were present in that from *Zea Mays* seedlings five days old. Also,

the exudate water from *Caladium antiquorum* gave a positive test with diphenylamine but no test with nitron.

Klein examined the first drop to appear on the leaves of *Boehmeria utilis* and *Fuchsia* sp., and found nitrates but no nitrites. The nitrites appeared in the exuded water after from six to eight hours, giving a strong test with Griess & Lunge reagents. After two days the nitrates had disappeared. Klein concludes that nitrites are found in the exuded water only after a partial reduction from the nitrates by bacteria or molds.

Lyon and Wilson (1921) grew plants whose roots were immersed in a sterile nutrient solution. They found in the solution surrounding the plant roots 535 milligrams of organic material, consisting in part of peroxidase and a reducing substance which were identified by color tests. This organic material had been liberated by the growing plant roots.

METHODS AND MATERIAL

It seemed desirable in the present study, because of the scarcity of material and the question of feasibility, to confine the investigation for the most part to a qualitative determination of various organic substances that may be present and readily detected in the exudate water. A number of tests were performed to determine some of the specific substances. Some of the tests were for the identification of specific organic compounds, while others were for inorganic substances. Klein considered that the nitrites which he found in the guttation water were produced from the nitrates by molds or bacteria. In order to avoid this complication, the various tests in this study were first performed on material collected from plants growing under non-sterile conditions, and then the technique was applied to exudate water collected from sterile plants.

The material for examination was collected from two sources. One was from maize grown in the greenhouse in soil. These plants were for the most part not more than three weeks old nor more than four inches high. They had from two to four blades when the water was collected. They were watered from an ordinary hose by spraying, but it is doubtful whether a great deal of this water was collected as exudate water. Exudate water was collected also from maize grown in water culture, and from the lawn grass, which was mainly blue grass, around the buildings on the campus.

The second source of material was from plants grown under sterile conditions. Among these plants were maize, timothy, and oats, grown from sterile seeds sown on a sterilized substratum.

From 20 to 30 cubic centimeters of the exudate water was collected in the course of an hour from maize which was growing under non-sterile conditions. This was taken from either the ends or the sides of the blades. It was used immediately in testing for certain substances which it might contain. In all probability it was somewhat more concentrated than when first exuded. All determinations were made, however, on the material as collected. Smaller amounts were collected from maize, oats, and timothy grown under sterile conditions.

PRESENCE OF CERTAIN INORGANIC MATERIALS IN WATER FROM HYDATHODES

Total solids

In making a determination of the inorganic as well as the organic materials in the exuded water, it was desirable to know the proportion of each. This was determined by evaporating 10 cubic centimeters of the water and weighing the residue both before and after igniting it. The material which was left after ignition was called *inorganic*, while that which was driven off on ignition was called *organic*. The results of three determinations are given in table 1:

TABLE 1. TOTAL SOLIDS AND ORGANIC MATTER IN WATER FROM HYDATHODES

Source of water	Total solids (parts per million)	Parts per million left after ignition	Parts per million lost on ignition
Non-sterile maize	1,030	280	750
Sterile timothy	573	377	196
Sterile timothy	220	90	130

These results indicate that there was a considerable variation in total solids of various collections; also, that the amount lost on ignition, which was called *organic matter*, varied from 130 to 750 parts per million. This variation may be due partly to the fact that plants of different ages were used.

Nitrites

To about 5 cubic centimeters of the exudate water from maize plants forty-three days old, the Griess reagents for the detection of nitrites were added. After a few minutes a pink color began to appear. At the end of twenty minutes the color was very pronounced but was much fainter than that of a standard which represented 0.0001 milligram of nitrites per cubic centimeter. The reagents did not give this test with distilled water.

In a second test, from three to four drops of an aqueous solution of 1.2 per cent sulfanilic acid was added to 4 cubic centimeters of the exudate water from maize plants forty-three days old, and the materials were mixed. From two to three drops of concentrated hydrochloric acid and an alcoholic diphenylamine solution was added to this mixture. When these were mixed, the red color that appeared was taken as an indication of nitrites. The reagents and distilled water did not give this test.

A third test for nitrites consisted in adding an alcoholic solution of alpha-naphthylamine and dilute hydrochloric acid to some of the exudate water, the development of a deep violet being considered a positive test for this constituent.

Exudate water from sterile maize, oats, and timothy, from eight to eighteen days old, gave these tests for nitrites.

Nitrates

To test for nitrates, 5 cubic centimeters of the exudate water from maize was evaporated to dryness, and to the residue was added 0.1 cubic

centimeter of phenoldisulphonic acid. The residue and acid was rubbed with a glass rod. After standing for ten minutes, 0.5 cubic centimeter of water was added, and then an excess of ammonia water 1:1. The development of a yellow color gave evidence of the presence of nitrates. This test was positive also with exudate water collected from maize eleven days old, oats nine days old, and timothy fourteen days old, growing under sterile conditions.

PRESENCE OF CERTAIN ORGANIC MATERIALS IN WATER FROM HYDATHODES

Reduction of methylene blue

The object of the first test for the presence of organic materials was to determine whether or not these materials would reduce methylene blue. The test was made in a white porcelain crucible. To 0.5 cubic centimeter of a normal solution of sodium hydroxide, enough methylene blue solution was added so that the bottom of the crucible was just visible. This mixture was then heated to the boiling point and some of the exudate water was added. With this procedure the color of the methylene blue entirely disappeared. On cooling, a color developed which had considerable red in it. This was considered a positive test for reducing substances. This reaction occurs when reducing sugars, and probably other substances, are present in the solution, and gives a positive test when 0.1 cubic centimeter of the solution being tested contains 0.0000039 milligram of glucose. This test was applied to exudate water collected under sterile conditions from maize, oats, and timothy, with positive results.

Sugar

The test for sugar was made according to the recommendations of Heriot (1920). It was positive with amounts as small as 0.004 per cent of sugar. To about 5 cubic centimeters of the water from maize, four drops of an alcoholic alpha-naphthol solution was added, and the two were thoroughly mixed. Concentrated sulfuric acid was added to form two layers. On standing, a bright red to deep violet color appeared at the surface of contact of the two liquids. The color became intense if the whole mixture was stirred and gently heated. Exudate water from sterile maize plants collected eleven days after planting, gave a positive test in less than two minutes. For exudate water collected from sterile oats nine days old, thirty minutes was required to produce the characteristic reaction. The test for sugar was positive with exudate water collected from sterile timothy plants varying in age from nine to eighteen days.

Enzymes

Catalase.— To about 9 cubic centimeters of the exudate water from maize, 0.5 cubic centimeter of hydrogen peroxide was added, and the solution was gently rotated to insure a thorough mixing. After a few minutes, bubbles began rising from the interior of the mixture. This action was accelerated when the mixture was warmed. No bubbles appeared in a similar mixture when distilled water and hydrogen peroxide were used. A similar determination with boiled exudate water gave

o bubbles. The bubbles were taken as an indication of the presence f catalase. The test was positive when made with exudate water colcted from sterile oats, maize, and timothy.

Peroxidase.— In making the first test for peroxidase, 5 cubic centimeters the exudate water from maize was placed in a test tube and about 0.1 bic centimeter of hydrogen peroxide was added. The solution was ixed thoroughly and allowed to stand for from two to three minutes. fter this interval a few drops of a five-per-cent phenol solution was added. peroxidase was present, a browning of the solution occurred and after time a precipitate settled to the bottom of the test tube. This reaction is very decisive. The browning began within ten seconds after the test is made, and was accompanied with a heavy brown precipitate. When similar test was made using boiled exudate water, no reaction was tained.

Tests with water from hydathodes of sterile maize eight days old and ts nine days old were also very decisive, while a test from timothy en days old was negative and one from plants ten and thirteen days l was positive.

In a second test for peroxidase, two drops of hydrogen peroxide was ded to about 3 cubic centimeters of the exudate water from maize, l the materials were mixed. In about one minute two drops of an oholic solution of guaiac was added. There was instantly a bluing the guaiac, the blue color becoming intense. This did not occur if lrogen peroxide was omitted or if the exudate water was boiled. This t was positive when made with exudate water collected from sterile ize, oats, and timothy.

Reductase.— Klein (1913) reports that no nitrites were found in water m the leaves of *Boehmeria utilis* and *Fuchsia* sp. when it was examined nediately after being excreted, but that they appeared after from to eight hours, and that on longer standing ammonia developed. is loss of nitrates and subsequent appearance of nitrites and ammonia in ascribed to the action of molds and bacteria. It would seem that re are other possibilities in such a reduction. Nitrate-reducing enzymes found in many plants, and, since exudate water has been in contact h living cells of the roots, the stems, and the leaves, it may have in he power to reduce nitrates to nitrites and ammonia.

n a test to determine the presence of reductase, exudate water was ected from sterile timothy plants eighteen days old. The materials e used in the following proportions: 10 cubic centimeters of exudate, ubic centimeters of a 50-per-cent solution of $NaNO_3$, 0.1 cubic centier of benzyl alcohol as an accelerator, 0.9 cubic centimeter of water. a control, the 10 cubic centimeters of exudate was replaced by boiled date water. These materials were placed in a stoppered container thoroughly mixed. The test and the control were kept at about C. for forty-eight hours. At the end of this time the presence of ites was determined with the Griess reagents. It is presumed that e conditions were not favorable for bacterial growth.

comparison of the solutions showed at least twice as much nitrite he test as was found in the control. A somewhat similar test conted for twenty-four hours, in which exudate water from sterile timothy

plants fourteen days old was used, also was positive though not so pronounced.

Tests of this character using water from maize fourteen and eighteen days old gave no increase in nitrites.

HYDROGEN ION CONCENTRATION OF WATER FROM HYDATHODES

It was pointed out by Haas (1920) that the hydrogen ion concentration in expressed sap of plants varies with the kind of plant, its stage of maturity, and the substratum on which it was grown. In this work it was desirable to know whether or not the exudate water of young plants would vary in hydrogen ion concentration in the same way. In order to throw light on this point, maize, oats, and timothy were grown under sterile conditions on the same kind of substratum, and timothy was grown also on five different kinds of substrata. The exudate water was removed from all the plants each day, and the hydrogen ion concentration was determined by the colorimetric method as published by Clark (1920), with the slight modification that the exudate water was placed in the wells of a spot plate and a small amount of indicator was added to each. The resulting colors were compared with Clark's color chart to determine their pH value. The findings are recorded in table 2:

TABLE 2. HYDROGEN ION CONCENTRATION OF EXUDATE WATER FROM MAIZE, OATS AND TIMOTHY

(Plants seven days old at time of first collection)

Number of days after first collection	Maize	Oats	Timothy				
	Substratum* 1	Substratum* 1	Substratum* 1	2	3	4	5
	pH	pH	pH	pH	pH	pH	pH
0	8.2	6.3	6.6	6.8	7.0	6.8	[illegible]
1	6.2	6.4	6.2	6.8	7.0	6.2	[illegible]
2	6.4	6.6	6.2	6.6	6.6	6.4	[illegible]
3	6.4	7.0	6.4	5.8	6.6	6.8	[illegible]
4	5.2	6.4	5.6	5.6	6.4	6.8	[illegible]
5	5.2	6.6	6.4	6.4	6.4	......	......
6	5.3	6.6	6.2	6.2	6.2	......	......
7	6.4	6.4	6.2	6.2	......	......	
8	5.6	6.2	6.2	6.2	......	......	
9	5.0	5.2	6.2	7.4	......	......	
10	5.0	6.2	......	......	......	......	
11	5.1	6.4	......	......	......	6.4	[illegible]
12	6.2	7.0	6.2	......	......	6.4	
13	6.2	....	......	6.9	6.2	......	
14	6.6	....	......	......	......		

* Substratum 1, full nutrient solution plus 1.5 per cent of agar to solidify; 2, distilled water and 1.5 per cent of agar to solidify; 3, soil with 30 per cent of full nutrient solution; 4, soil with 30 per cent tap water; 5, soil with 30 per cent of distilled water.

It is observed that the first water exuded from the hydathodes of young maize, oats, and timothy plants as measured by the colorimetric method was approximately neutral and that as the plants became older the exuded water became more acid. In the case of maize this acidity increased

ntil it was about the same as that of the expressed sap of much older lants as determined by Haas. The reaction of the water from maize, mothy, and oats grown on a similar medium was not the same; the water om maize became considerably more acid than that from timothy or ats.

The hydrogen ion concentration of exuded water from timothy plants rown on a number of substrata suggests that with young plants the ibstratum makes very little if any difference in the hydrogen ion conntration. Probably the temperature and light conditions also are factors hich operate to change the hydrogen ion concentration.

WATER FROM HYDATHODES AS A MEDIUM FOR BACTERIAL GROWTH

In a test to determine the extent of bacterial growth on water from hydaodes, 0.002 cubic centimeter of the exudate water from non-sterile maize is spread over 1 square centimeter of surface on a microscopic slide. ter the water had spontaneously evaporated, the slide was passed rough a flame and the residue was stained with Ziehl-Nielson carbolchsin. On examining this under the microscope it was observed that e natural contamination of the material as collected was less than 0 bacteria per cubic centimeter. Some of this exudate water was ubated for forty hours at room temperature, and the organisms then sent were again determined. By that time such a heavy growth of cteria had developed that the solution was very cloudy and an examinan similar to the preceding showed more than 100,000,000 bacteria per bic centimeter. A series of plates were made in order to determine number present by this method. The plate counts showed more in 90,000,000 bacteria per cubic centimeter. A part of one colony was nsferred to a slope and used subsequently in determining the growth the organism in sterilized exudate water from lawn grass (table 3, H). Vater was collected from grass growing around the buildings on the npus. This was filtered through paper after the paper and the funnel l been thoroughly washed with distilled water and drained free of ess water. It was then distributed into carefully cleaned test tubes. ilized, and inoculated with certain bacteria, the growth of which determined. The result is given in table 3:

LE 3. GROWTH OF BACTERIA IN HYDATHODE WATER FROM GRASS ON CAMPUS LAWN

(Incubated at 25° C.)

Organism	Number of bacteria introduced per cc.	Bacteria per cc. 24 hours later	Bacteria per cc. 48 hours later
ite reducer	19,500	44,500,000	103,000,000
lus *cereus*	65	1,750,000	900,000
orescens	2,500	8,000,000	16,000,000
dicicola *	835	850,000	513,000
	19,000	87,000,000	146,000,000

st plant, *Trifolium repens.*
organism isolated as natural contamination of water from hydathodes of corn.

The data show that there was a large increase in bacteria per cubic centimeter in twenty-four hours. A further increase is evident with three of the organisms after forty-eight hours. The falling-off in numbers with the other two organisms at the forty-eight-hour period is probably due to their having used all the organic material suitable for growth.

DISCUSSION OF RESULTS

The work herein reported shows that in the exudate water from the hydathodes of maize, oats, and timothy, both inorganic and organic materials were found. This was observed in the water from plants varying in age from eight to forty-three days. Color tests were made to identify some of the compounds. Since it is recognized that infection by molds or bacteria might change the composition of the water in a short time, the exudate water was collected from sterile and non-sterile plants for examination. The total solids were determined by evaporating a definite amount of the water and weighing the residue. The weight which was lost when the total solids were ignited was considered organic matter. No effort was made to determine what salts were present in the water other than nitrates and nitrites. This has been determined in a measure by other workers.

The organic materials that have been identified suggest that the exudate water may have a similar composition to that of the plant sap. This supposition is especially warranted by the fact that the exudate contains several enzymes which are known to be present within the sap and that the hydrogen ion concentration is almost the same as that of expressed sap of similar plants as reported by Haas.

The identification of a substance capable of reducing nitrates to nitrites suggests that the nitrates which are taken up by the plant from the substratum are in part reduced to nitrites as they pass up through the plant tissues, and that this reduction may continue for some time after the water has been exuded through the hydathodes.

The organic material that is present in the water from hydathodes seems to be easily utilized by bacteria. Since under field conditions this water finds its way into the soil, it must serve similarly as a temporary source of food for soil organisms.

SUMMARY

The chief points brought out in this paper are the following:

Total solids in the water exuded through the hydathodes from maize plants growing under non-sterile conditions were as high as 1030 parts per million. The total solids in water from timothy plants which were growing in closed containers in the absence of microorganisms were much less, being in one case only 573 and in another only 220 parts per million. In all cases the total solids were more than half organic matter.

Reactions were obtained which indicated the presence of nitrites, nitrates, materials capable of reducing methylene blue, catalases, and peroxidases, in the exuded water from maize, oats, and timothy. Reductases were probably present in the water from timothy, but no reaction was observed to indicate their presence in the water from maize.

The exuded water from various plants was a good medium for the owth of bacteria. This was evidenced by an increase in the number bacteria in inoculated water.

The hydrogen ion concentration of water from hydathodes of maize, ts, and timothy is nearly neutral when the water is exuded by young ants. The acidity increases as the plants become older, until a maximum is obtained.

CONCLUSION

From the data presented it seems logical to conclude that the water om hydathodes of plants contains both inorganic and organic materials, d that it is a good medium for the growth of certain soil organisms.

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MEMOIR 66

CORNELL UNIVERSITY

AGRICULTURAL EXPERIMENT STATION

SIMPLIFIED APPARATUS AND TECHNIQUE FOR THE ELECTROMETRIC DETERMINATION OF HYDROGEN ION CONCENTRATION IN MILK AND OTHER BIOLOGICAL LIQUIDS

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ITHACA, NEW YORK
PUBLISHED BY THE UNIVERSITY

MPLIFIED APPARATUS AND TECHNIQUE FOR THE ELECTROMETRIC DETERMINATION OF HYDROGEN ION CONCENTRATION IN MILK AND OTHER BIOLOGICAL LIQUIDS

Frank E. Rice and Arthur J. Rider

A great variety of forms of apparatus have been recommended for e determination of hydrogen ion (H+) concentration. Many of these e more elaborate than is necessary for ordinary purposes, and the ocedures recommended for making the measurement are often renered tedious with unimportant precautions. This is due partly to the ct that the influence of various factors on the accuracy and speed of e determination have not generally been known, very few systematic ies ever having been made from that point of view.

or purposes of convenience the apparatus for determining H+ conration can be considered essentially as of two parts. One includes standard cell, galvanometer, slide wire resistance, source of current, l all wiring connections. This part of the potentiometer system is ry well standardized, and the forms offered by the various manuurers differ only in slight details. The assemblage and manipulation his part of the system is so simple that there is practically no chance error. The other part of the equipment, however — the glass parts, uding the calomel cell, the hydrogen electrode cell, and the contions — is very much more sensitive. It is here that manipulation extremely important. While the potentiometer system has been in mon use for a long time, it is only comparatively recently that it s been very widely used for the measurement of H+ concentration; nsequently, it is the electrode vessels and appurtenances which are s standardized and upon which less comparative work has been done. When the writers started H+ concentration work, a number of forms apparatus were procured and the procedures recommended by ious investigators were tried. So much difficulty was found, and, the whole, such poor results were obtained, that it was concluded a ical study should be made of the various recommendations of pres investigators as to apparatus and means of measuring H+ contration. Gradually the apparatus and the procedure described in s paper were evolved, and they have for some time proved satistory in the hands of many operators.

PURIFICATION OF THE MATERIALS

The method suggested by Moseley and Myers (1918) was generally d for the preparation of pure water. Mercury was purified by istilling *in vacuo* several times. The method used for preparing e calomel was that recommended by Clark and Lubs (1916). The

product was preserved in a dark place and kept covered with 0.1 N KCl. The purest KCl obtainable was recrystallized several times for use.

As a plentiful supply of hydrogen gas is necessary, particularly for the apparatus here to be described, it was found best to make use of cylinders containing the gas compressed. No doubt the purity of the gas will vary with the method of its original manufacture. The particular consignment which was used in this experimental work was found to be good; identical results were obtained whether or not the gas was washed before it reached the hydrogen electrode vessel. However, washing the gas is very simple, and it is probably wise to always take the precaution. In this work the gas was passed from the cylinder through successive wash bottles containing, respectively, alkaline pyrogallol, dilute sulfuric acid, mercuric chloride solution, and water, and finally through a calcium chloride tube containing dry cotton.

THE CALOMEL CELL

The essentials of the calomel cell are: a wire in contact with the mercury layer, leading outside; above the mercury, a standard KCl solution which is saturated with HgCl; then an arrangement, usually a siphon tube, to make contact between the KCl-HgCl solution and the remainder of the chain.

Cells containing normal, tenth normal, and saturated KCl are in common use. The tenth normal electrode is perhaps employed most generally; for one reason, among others, because the potential changes less with temperature. That electrode is the one used in this work.

The form of the vessel for the calomel electrode is unimportant. Most of those that are on the market are suitable, though it was found desirable to have a stopcock in the siphon tube such as is provided in the Clark calomel cell. The cell should be closed, in order to prevent changes in concentration of KCl which might result from evaporation.

The Clark cell and the Kelly cell obtained from the manufacturer were tested, together with a cell which was prepared in this laboratory. The last-named is illustrated in figure 1(A). It is simple in construction and was found to give results comparable with those obtained with the other two cells. In preparing this cell, a short piece of platinum wire is soldered to the end of copper wire. This is thrust through a glass tube until the platinum protrudes at one end, when that end is sealed in a flame. The tube is supported by a rubber stopper in a wide-mouth bottle of from 100 to 200 cubic centimeters capacity. In the apparatus used in this work, *a* and *b* were glass stopcocks, while *c* was a rubber connection with a spring pinchcock; but it would be possible at all these points to use rubber connections with pinchcocks.

In preparing the calomel cell, the vessel is carefully cleaned and dried. Mercury is placed in the bottom so that the wire will be covered. A paste is made up of the purified calomel with a little mercury, moistened with 0.1 N KCl solution. This is run in over the mercury until there is a layer about five millimeters thick. Then there is added

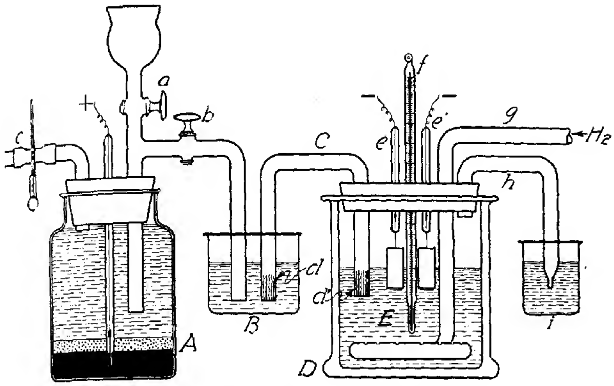

FIG. 1. APPARATUS FOR DETERMINATION OF HYDROGEN ION CONCENTRATION

N KCl solution which has been previously saturated with calomel. re must be taken throughout this operation that the mercury layer not disturbed, so that no drop of moisture will be left between the tinum wire and the mercury. A good contact at this point is neces-ry.

The cell should not be used for about two days after preparing, in ler to give opportunity for equilibrium to become established.

The upper part of the vessel and the siphon tube should be occasion-y flushed out, fresh KCl solution previously saturated with calomel ing used. This is to prevent the interior of the cell from becoming ntaminated through diffusion from the outside. It may be accom-ished by filling at *a* and opening *b* and *c* for drainage.

The stopcock *b* at the siphon tube is ungreased and is kept closed ring readings. This will prevent movement of the liquid through phoning action, and also lessen the chance of contamination through ube from the outside. At the same time, sufficient contact is made gh the liquid film standing around the stopcock for a free flow ctric current.

THE HYDROGEN ELECTRODE

hydrogen electrode consists of a sheet of metal, coated uniformly spongy platinum, palladium, or iridium, which is suitable for bing hydrogen gas at its surface. Some workers have used glass hich, by a special process, platinum is deposited.

d has been recommended by some investigators for the preparation ctrodes, it being stated that with this metal a perfect saturation drogen is more readily obtained, and, as a result, equilibrium s more quickly. Both gold and platinum were tried in this work,

but the latter was found the more satisfactory. Two gold and two platinum electrodes were platinized similarly and H+ concentration measurements made in 0.001 N HCl. Readings were taken from time to time until equilibrium was reached. Fourteen determinations were made with gold electrodes, and the average time required to reach equilibrium was found to be 12.5 minutes. On the same solution twenty-four determinations were made with platinum electrodes, with an average time to reach equilibrium of 7.3 minutes. It is seen that equilibrium was reached less rapidly by the use of gold electrodes than with platinum. The former has the disadvantage also that aqua regia cannot be used in cleaning. This reagent is particularly efficient in cleaning platinum black from electrodes. For these reasons the gold electrodes were not used in this work, but platinum was used exclusively.

The electrodes are prepared as follows: A piece of platinum foil cut, about 1 to 2 centimeters square. Platinum wire is welded to the foil by laying the wire and the foil in position on a block of Alberene Stone or any stone that can conduct the heat away but slowly. A very small flame from a blast lamp is directed at the point to be welded, and when the platinum is at red heat a sharp blow with a light hammer is sufficient to make the union. The platinum wire should be from 10 to 15 centimeters in length, or an economy may be effected by using a piece of wire from 2 to 3 centimeters long and soldering to this a piece of copper wire. The wire is then pushed into a glass tube so that only platinum protrudes at the foil end, and this end is sealed in a gas flame.

The surface of the platinum is cleaned by immersing it in aqua regia or chromic-acid mixture and then rinsing it with water. Platinum black is deposited on the foil by making it cathode in a 3-per cent solution of chlorplatinic acid containing a trace of lead acetate — the lead acetate causing the platinum black film to adhere better than it would otherwise. Somewhat better results were noted when at intervals the direction of the current was reversed, thus having the foil as anode. In this way two electrodes can be platinized together.

When the surface of the platinum appears to be well covered with platinum black, it is removed from the solution and washed.

The size and shape of the platinum foil have been found not to influence the results, with one exception. An electrode consisting of a small piece of platinum wire protruding from the support, similar to that described by Bovie (1915), at times gave results at variance with those from electrodes of larger surface exposure. There is a particular advantage in using the larger electrodes, in that, because of the size, the cell offers less resistance to the passage of current, and the whole apparatus is in consequence more sensitive.

Electrodes should not be allowed to become dry after preparation for use, but should be kept immersed in water or weak sulfuric acid solution.

Electrodes that have been used in fluids containing fats and proteins soon become useless. In some cases they can be regenerated by soaking in chromic-acid cleaning mixture, though frequently it is necessary

clean them entirely and replatinize. Washing in warm aqua regia will remove the platinum black coating, after which the electrodes are ready for replatinizing.

In preliminary work some use was made of the electrode vessel esigned by Clark (1915) and electrodes of the type suggested by ildebrand (1913). A modification of the Hildebrand electrode was lso prepared, with the tube supporting the platinum foil made to slide p and down in the outer shell. It was then possible to adjust the osition of the foil so that there would be a maximum exposure of the oil to both the hydrogen gas and the solution.

It has been found in this work that it is very desirable to make readngs from time to time for the calculation of the H+ concentration, in rder to be certain that the equilibrium point has been reached. The ime required for obtaining equilibrium varies with the type of solution, and in some cases is very long. With the Clark vessel it is impossible to judge, except by making a number of separate determinations, when the equilibrium point is reached, since after one reading is made it is necessary to empty the apparatus and begin again. This is the chief objection to this cell.

In using electrodes of the Hildebrand type it was found that equilibrium was reached extremely slowly; in some samples of milk, ubbling hydrogen through the liquid as long as forty-five minutes was necessary before a constant reading could be obtained. This was elieved to be due to the fact that with this type of electrode the surface of the liquid is in contact with air. Any type of electrode vessel hich is so enclosed as to maintain an atmosphere of hydrogen over he liquid would eliminate this objection.

FACTORS INFLUENCING THE RAPIDITY WITH WHICH EQUILIBRIUM IN READINGS CAN BE REACHED

The literature on H+ concentration determinations, and also laborary experience, emphasize the desirability of finding an apparatus and method which will bring about certain equilibrium with the least posle delay. Some investigators have reported that even several days ere necessary at times for obtaining constant results. The most portant points in this regard relate to the hydrogen electrodes and e design of the vessel. The calomel electrode, from a few days after eparation, remains constant for months. In some cases, after equibrium has been reached a slight drift in potential is noticed, which ay be due to diffusion at the surfaces between the solutions of different ncentrations in the chain; but this can be counteracted by providing esh surfaces each time a reading is to be taken, and finishing each easurement as rapidly as possible. The only other cause for variation potentials pertains to the hydrogen electrode and the liquid bathing Correct potential is not obtained at this point until time has been en for the following general conditions to be reached: pure hydrogen exclusively must be adsorbed on the surface of the spongy platinum;

all foreign gases, especially oxygen, must be swept out of the liquid or reduced by the hydrogen at the surface of the platinum; substances capable of taking up hydrogen must be saturated with it; time must be given for diffusion of the liquid into the pores of the spongy metal.

A preliminary study was made of these factors by employing Hildebrand electrodes in various ways. Each condition and method of manipulation which was thought to affect the time of reaching equilibrium was taken up, with the end in view of finding those which would reduce the time to a minimum. The results are given in the following paragraphs.

Effect of depositing hydrogen on the platinized platinum foil by electrolysis

Loomis and Acree (1911) suggested placing the electrodes in a 2-per cent H_2SO_4 solution and making them cathode in a circuit. In this way hydrogen is liberated at the platinum black surface and a considerable adsorption of the gas takes place, with the result that less time is necessary for saturation of the electrode later, when it is immersed in the solution, and correspondingly less time should be consumed in reaching equilibrium of readings.

On determining the hydrogen ion concentration of 0.001 N HCl four determinations were made, electrodes that had had hydrogen plated out on them, as just described, being used. Five, six, ten, and fifteen minutes, respectively, were required to obtain equilibrium in readings. Four determinations with electrodes not so treated required twenty, twenty, forty, and forty minutes, respectively.

Effect of passing gaseous hydrogen over the electrode

Robertson (1907) recommended that in preparation of hydrogen electrodes they be immersed in water, and hydrogen gas bubbled over them for some time.

In some measurements which were made on 0.001 N HCl, all the electrodes were prepared in exactly the same way, except that some were treated by immersion in this solution and bubbling hydrogen over them for a considerable period of time. An average of eight determinations when the electrodes were not so treated, showed that 10.9 minutes was required to reach equilibrium, and the average of ten determinations for those treated was 5.2 minutes.

These experiments show distinctly that the time required in reaching equilibrium of readings is due partly to the slowness with which the electrode itself is saturated with hydrogen, and that time is saved if they are given these preliminary treatments.

Effect of previously saturating the solution with hydrogen

As was pointed out by Konikoff (1913), oxygen or oxidizing substances in the solution are detrimental to the measurement of H-con

ntration because of a reducing action taking place at the surface of e electrode. Also, the presence of any gas other than hydrogen lowers e hydrogen pressure, and as a result an incorrect potential is observed. ecause of this, it is important that any foreign gases be swept out d that any oxidizing substances be reduced, before accurate results n be obtained.

In making sixteen determinations on 0.001 N HCl, in half of which e samples had been previously treated by bubbling hydrogen gas rough them, it was found that on the average 6.3 minutes was required r the samples which had been so saturated and 15 minutes was quired for those which had not, all other conditions of the deterinations being the same.

It is here seen that not only is saturation of the electrode necessary fore equilibrium can be reached, but also saturation of the solution th hydrogen.

Effect of exposing the electrode alternately to the atmosphere of hydrogen and to the liquid

t has been suggested by Clark (1915) that in order to reach equiium rapidly the H+ cell should be so built that the entire electrode bathed alternately with hydrogen gas and with liquid. This has n made a special point also in the construction of the Bunker (1920) etrode.

n experimenting with the Hildebrand electrode and 0.001 N HCl, rty-two determinations were made in which the electrode was raised hand into the hydrogen and lowered into the liquid alternately until ilibrium was reached; it was found that, as an average, 8.4 minutes s required for equilibrium. Thirty-two determinations were made n with similar electrodes without attempting to regulate the rise and of liquid over the electrode. In some of these cases the time ired for equilibrium was less than the above-named average; how- the average of all determinations was 12.3 minutes.

is seen that a definite advantage is gained by so handling the Hildend electrode that it is bathed alternately with liquid and gas. In t of the electrodes designed to accomplish this result, a mechanical king or rocking device is used. However, it is believed that the e result can be obtained much more easily by admitting a rapid am of hydrogen into the liquid immediately below the electrodes. s scheme is used in the hydrogen electrode vessel eventually pred for this work, which is described later. Similar results are ined with the electrode vessel designed by Loomis and Acree 1).

Effect of shaking the electrode vessel

e use of electrode vessels equipped with shaking devices has been mon. It has been believed that as a result of shaking there is a rapid interchange between the solution, the gaseous hydrogen, and electrode, also that any molecular or combined oxygen in the solu-

tion is more quickly brought into contact with the electrode ar reduced.

Throughout the course of these experiments it has been observe that it is of decided advantage to keep the liquid in vigorous agitation either by shaking the electrode vessel or by the rapid bubbling c hydrogen through the solution. So far as could be seen, neither c these methods has any particular advantage over the other in establish ing equilibrium rapidly. Since a mechanical device is necessary i shaking of the electrode vessel is to be a part of the procedure, it wa omitted in the apparatus which was finally adopted, and agitation wa brought about by a very rapid bubbling of hydrogen gas entering a the bottom of the liquid.

AN IMPROVED HYDROGEN ELECTRODE CELL

Individual hydrogen electrodes occasionally give incorrect results, sometimes through intimate adsorption of impurities, commonly called *poisoning*. Also, particularly after being used in biological liquids, the surfaces may become coated with fats, proteins, or other organic sub stances, so that abnormal voltages are yielded. It is therefore desirab to use more than one electrode as a check on results.

In the cell eventually assembled for use in this laboratory, it is po sible to include as many as six electrodes, and merely by switching fro one to another, readings can be taken on all without delay.

This cell (figure 1, D) consists of a glass cylinder about 7 centime in diameter and 10 centimeters deep, fitted with a rubber stoppe through which are holes admitting the electrodes (e and e'), a ther mometer (f), a hydrogen inlet tube (g), a hydrogen outlet tube (h) and a siphon tube (C) connecting the body of the liquid with th reservoir (B), which in turn is in contact with the liquid in the calome electrode vessel.

The hydrogen inlet tube terminates in a horizontal ring which i perforated to give a large number of openings so as to distribute th flow of hydrogen through the body of the liquid. Thus a rapid a complete saturation of both liquid and electrodes is effected.

The hydrogen outlet tube may dip into a reservoir of water thus acting as a water seal and securing an atmosphere of hydrog throughout the interior of the cell by preventing an access of ai However, as long as the hydrogen is flowing freely there is no oppo tunity for the entrance of air, and the use of the water seal is not th necessary.

Assembling the apparatus

Making contact between the mercury and the hydrogen electrodes

Calomel cells and hydrogen electrode cells must be joined by m of a solution which is highly conducting, since for maximum s tivity there should be offered no more resistance than is necessar

e passage of the current. A saturated solution of KCl is ordinarily nployed, and that was used in this work. The siphon tube *C* was led with this solution, and the reservoir *B* with 0.1 N KCl. The be *C* is of about five millimeters internal diameter and is plugged at e ends with small rolls of filter paper, which prevent a loss of the juid when the tube is raised, and also lessen diffusion. This tube ould not fit so tightly in the rubber stopper but that it can be raised d lowered easily.

This assemblage is designed to reduce contact potential to a minimum, nich is a possible source of error depending on the fact that a tential is set up between two liquids of dissimilar concentration at ir juncture. For example, an electromotive force is set up between saturated KCl and 0.1 N KCl solutions, and also, on the other side the chain, between saturated KCl and the liquid in the hydrogen l. For extremely accurate work this is taken into account and calated by the classic method of Bjerrum (1905). However, it has been und by the same investigator that this source of error is reduced to vanishing point by the use of saturated KCl as previously described, the differences of potential at the liquid junctions at the two ends the saturated KCl solution are about the same. Since the potential ferences are in opposite directions, they tend to equalize each other.

uipotential shielding of the apparatus

There were periods during the investigation when it seemed impos- le to obtain results of any regularity, this difficulty seeming to come efly in damp weather. On the assumption that the trouble was due stray electric currents, the suggestions of White (1914) were fol- ed to insure protection against any such effects. The desk top was ered with a large sheet of aluminum. Then under each piece of paratus was placed a piece of glass. Thus each instrument was ulated from every other one except through the proper connections, l the whole system rested upon an equipotential base which shielded from stray currents. No further trouble of this nature was ountered. As a precaution, shielding should never be omitted.

Making the determination of hydrogen ion concentration

After the sample on which determination is to be made is placed in hydrogen electrode vessel, the electrodes should be adjusted so t the foils are about half submerged. Careful inspection should be le of the siphon tube *C* and the tube leading out of the calomel cell, nake sure that no air bubbles are present, in order that the contact be perfect throughout the chain.

he proper connections are made from the mercury and the platinum s to the potentiometer. The use of knife switches makes convenient change from one hydrogen electrode to another. A rapid flow of rogen is maintained throughout.

The siphon tube *C* should be raised except while the readings are being made. After a few minutes the first readings are taken, and readings are continued from time to time, first with one electrode and then with the other, until there is no longer any change. The hydrogen need not be shut off while the measurements are being made. The time at which equilibrium is reached depends upon many things, particularly the nature of the sample. With milk, twenty minutes is sufficient, though it is possible that there are other biological liquids which require a longer time.

Generally, no trouble was experienced from frothing, either with raw milk or with milk reconstituted from evaporated and condensed milk. Powdered milk from a large number of manufacturers was studied, and in but one instance was any difficulty experienced in this regard, and it was not then impossible to obtain results. It may be noted that the use of octyl alcohol has been found satisfactory by Schmidt (1916) on such occasions.

In addition to being able to check hydrogen electrodes one against the other, it is also nearly as desirable to have results from more than one calomel cell. Any number of calomel cells can be prepared, two or more can at the same time dip into reservoir *B*, and readings can be taken from all for comparison.

Readings are made on the potentiometer in volts or millivolts, and it remains then to calculate H^+ concentration from these results. Tables given by Schmidt and Hoagland (1919) were made use of in this work.

Correction for temperature

Most methods of calculation and tables are designed for measurements to be made at 25° C. Many investigators enclose the apparatus in an air bath or a bath of liquid, and thus hold the temperature constant at 25° or any other point desired. The use of constant-temperature rooms adds a considerable expense and trouble to the manipulation, and the immersion of the whole apparatus in a liquid is liable to cause errors through current leakages. On the other hand, it has been found in this work that the temperature correction methods suggested by Schmidt and Hoagland do not give good results.

The method which was finally adopted and which yielded satisfactory results was to hold at 25° C. only the sample on which the H^+ concentration measurement is to be made. This can easily be done by immersing the hydrogen electrode vessel in a water bath. By observing the thermometer which dips into the sample, the temperature of the sample can be brought to the desired point.

While the hydrogen electrode is very sensitive to a variation of a single degree of temperature, the potential of the calomel cell changes but little with small variations, and it was believed unnecessary to maintain it at a constant temperature. However, it is best not to allow the temperature of the calomel cell to vary too widely from 25°. The room should be not below 20° C.

FECT ON DETERMINATIONS OF HAVING ELECTRODES WHOLLY OR PARTLY IMMERSED IN THE LIQUID

Clark and Lubs (1916) make a point of the fact that readings were iken with the electrode wholly immersed in the liquid. McClendon and harp (1919) state: "If the solution to be tested is sufficiently viscous support a layer of the solution on the platinized electrode (as in the se of blood plasma), it is not necessary to take the reading with the trode totally immersed."

ome experiments were made on milk, measuring the potential with electrodes wholly immersed and with the electrodes only half nersed, and it was found that if the electrodes were small — less than ut one centimeter square — there was generally a difference of from to 3 millivolts, the electrodes wholly immersed giving the higher lues. With very large electrodes no differences were noted. At st one square centimeter of foil should be immersed in the liquid at time of making the reading.

It will be remembered that in the manipulation of the H+ apparatus eviously described, it is recommended that the readings be taken with e full flow of hydrogen passing through. Even though the electrodes e only about half submerged when the liquid is at rest, the continual bbling of the gas passing through the liquid below the electrodes eps the entire surface of the foil well covered, and the same effect produced as though the entire electrodes were really beneath the rface.

RESULTS OBTAINED WITH THE USE OF THE HYDROGEN ELECTRODE EQUIPMENT HERE DESCRIBED

On standard buffer solution

lutions of known H+ concentration are often used in checking the vior of H+ concentration apparatus. A standard solution of acid ssium phthalate was made up according to Clark and Lubs (1916), h has a P(H) value of 6.711. The following results were obtained six hydrogen electrodes, running two at a time, five determinations g made with each pair:

Determination	Electrode					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
....	6.696	6.696	6.708	6.708	6.708	6.708
....	6.707	6.707	6.704	6.704	6.704	6.704
....	6.712	6.712	6.702	6.702	6.722	6.722
....	6.712	6.712	6.708	6.708	6.698	6.698
....	6.717	6.717	6.710	6.710	6.713	6.713
erage..................	6.709	6.709	6.706	6.706	6.709	6.709

ese results, obtained by the use of the apparatus here described, the variations (1) between different electrodes, (2) in making

different determinations on the same solution, and (3) between the true values and those obtained by the apparatus.

On samples of raw milk and manufactured milk reconstituted with water

Determinations totaling 459 were made on samples of raw milk, also on evaporated and powdered milk reconstituted with water according to the manufacturers' directions. In each determination, readings were taken using hydrogen electrodes in pairs. In 444 cases identical results were obtained with the two electrodes. In the remaining 15 cases the differences varied from 0.0004 to 0.0010 volt, which represented a variation in P(H) value of about 0.007 to 0.017. The samples ranged from 6.543 to 7.022.

EFFECT OF CARBON DIOXIDE UPON HYDROGEN ION CONCENTRATION

As has been pointed out by Clark (1920), in solutions with P H values above 5 the presence of carbon dioxide becomes of more and more importance. In the determination of H^+ concentration by use of the apparatus here described, this gas would of course be driven out of solution. If carbon dioxide is present in a nearly neutral liquid, and it is desired to measure acidity due to all factors including this (as in the case of blood, mineral water, and so on), then the apparatus here described should not be used. Under such conditions an electrode vessel of the closed type should be employed.

Except in a few cases such as in blood studies, however, carbon dioxide is not a part of the intricate physical chemical arrangement of the various soluble constituents which are important in physiological investigations. A soil solution, for instance, with a low P(H) value due to carbon dioxide, might be an extremely fertile soil, since carbon dioxide has a highly solvent action on the mineral matter in soil; on the other hand, an equally low P(H) value due to some fixed soluble acid might be extremely detrimental. Carbon dioxide is present in considerable quantity in freshly drawn milk, and it perhaps influences the H^+ concentration slightly. However, acidity due to this gas can not be of any importance in manufacturing operations, since the gas would soon be driven out, nor is it likely to be worthy of consideration in market milk. Also, in any purely scientific investigation of milk wherein H^+ concentration determinations are studied, whatever slight effect the presence of this gas may have should be disregarded.

The apparatus for H^+ concentration determination here described is therefore recommended for experimentation on all biological liquids except in those few cases in which carbon dioxide is of prime importance as such.

HYDROGEN ION CONCENTRATION DETERMINATION WHEN THE AVAILABLE AMOUNT OF SAMPLE IS SMALL

There are occasions in biological work when only a small amount of sample can be obtained. In such cases it is necessary to use a hydro-

ctrode cell of small dimensions. While cells used in this work conned as many as six hydrogen electrodes, and all parts were of liberal portions, by which means it was believed that the most nearly accue results could be obtained, it would be possible to reduce the size siderably in every way and use only one or two hydrogen electrodes. careful manipulation of such an apparatus entirely reliable results 1 be obtained.

ADAPTATION OF THE APPARATUS FOR ELECTRO-TITRATION

enerally in electro-titration, results of any considerable degree of uracy are not obtained. Vessels are used which are open to the air, l the designs are such that a satisfactory saturation of the hydrogen ctrode cannot be carried out.

By merely providing another hole in the stopper of the apparatus e used, and the introduction of a burette, electro-titrations can be dily made. Since the hydrogen gas is admitted rapidly at the lower ers of the liquid, there is sufficient agitation so that it is not necesy to provide a mechanical stirrer.

SUMMARY

An investigation has been made of the influence exerted by certain iations in procedure upon the speed and accuracy of the determina1 of H+ concentration. With the knowledge thus gained, an paratus has been designed which is simple of construction and easy l rapid of manipulation.

The apparatus is designed so that several hydrogen electrodes can checked against one another, as well as comparisons made between erent calomel cells. One reading after another can be made on a en portion of liquid, thus determining the equilibrium point without nging samples.

A simple yet accurate means of temperature control is suggested.

This apparatus is well adapted for use in student laboratories, as l as in research work; and merely with the introduction of a burette, ctro-titrations can be made more nearly accurately than by the paratus ordinarily employed for that purpose.

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AGRICULTURAL EXPERIMENT STATION

OBSERVATIONS ON THE LIFE HISTORY OF TAPHROCERUS GRACILIS (SAY) (BEETLE, FAMILY BUPRESTIDAE)

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BSERVATIONS ON THE LIFE HISTORY OF TAPHROCERUS GRACILIS (SAY) [1]

(BEETLE, FAMILY BUPRESTIDAE)

ROYAL N. CHAPMAN

The bulrush leaf miner, *Taphrocerus gracilis* (Say) (Say, 1825), belongs o that minority of the family of metallic wood-boring beetles, Buprestidae, ich mine in the leaves of plants. The eggs and the larvae have not been cribed in literature heretofore, and the life history has never been blished. So far as is known, this beetle is unique among the Buprestie in that it emerges and feeds for a month or two before it hibernates the adult stage.

This species is abundant in the vicinity of the Cornell Biological Field ation, and it was here that the present study was begun under the ection of Dr. James G. Needham.

DESCRIPTIONS OF STAGES

The adult beetles

e adult beetles are shown in Plate II, 3 and 4. They vary in length 3 to 4.7 millimeters. A large number of specimens from Okefenokee np, Georgia, are uniformly small, measuring only about 3 millimeters ngth. Those from central New York and southern Minnesota are neral uniformly large, altho a few specimens have been taken which early as small as those from Georgia.

e beetles are flattened in form, and the general contour is smooth. have grooves into which the appendages fit when retracted, and these so formed that when the legs and the antennae are retracted into n the ventral surface of the body is hardly less smooth of contour the dorsal surface. The antennae fit into grooves which extend ro-ventrad along the sides of the head and the prothorax. The pro- acic legs fold with the tibiae anterior to the femora, while the other legs with the tibiae posterior to the femora. The tarsae fit snugly into ves about the coxae.

The egg

e egg of this beetle (Plate I, 1) is oval in outline and measures 0.067 .05 millimeters. When first deposited, it appears fluid-like and trans- nt. In a few minutes it becomes whitish, and after the lapse of a hours it is shiny black. When deposited, it flattens out on the leaf a drop of viscid fluid with the margins extremely thin and the center t half a millimeter in thickness. Around the margin of the egg there transparent substance which adheres so closely to the leaf that some e epidermis of the leaf is often torn away when the egg is removed. is evidently a mucilaginous substance secreted by the female to serve

[1] ntribution from the Entomological Laboratory of Cornell University.

the purpose of gluing the egg to the leaf. The egg membranes remai attached to the leaves thruout the season, and even in the fall may b found adhering to the deserted blotch mines.

The larva

The larva varies so greatly, from the first instar to the last, that it i at first difficult to recognize it as the same species in the different instars When the larva is first hatched (Plate I, 2), the prothorax, into which th head is retracted, is very broad in proportion to the rather slender abdomen, and the general appearance is like that of the buprestid larvae which burrow in the wood (Burke, 1917). At each side of the head, a small, clublike appendage projects from the prothorax. These append- ages are enlarged at their distal ends and are covered with a very thin, spinous layer of chitin. The larva is able to retract the appendages to some extent, and seems to use them while feeding, as is described later.

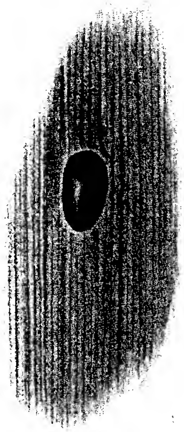
The size of the head, as determined by measuring to the outer margins of the longitudinal apodemes (Plate I, 2), has been found to be uniform within each instar. The head of the first instar measures 0.01 millimete and that of the second instar 0.14 millimeter.

In addition to its larger size, the larva of the second instar (Plate I, 3 differs from that of the first in the smaller proportionate size of the pro- thorax, a difference which is due to the relatively smaller head which is less completely retracted into the prothorax. Whereas the prothorax at the beginning of the first instar is approximately twice the width of the remain- ing segments of the body, which are all of about uniform width, the body of the second-instar larva gradually tapers from the rather broad prothorax to the terminal, or tenth, abdominal segment, which is the narrowest the series. A rectangular chitinous shield is developed on the median ar of both the dorsal and the ventral side of the prothorax.

Even within each instar there is a noticeable difference with regard t the retraction of the head. During the first part of each instar, the hea is larger in proportion to the remainder of the body, and is usually mor retractile; but later, after the body has grown, the head is proportionatel smaller and somewhat less retractile.

The larva of the third instar (Plate I, 4) differs from the second ma in size, the head measurement being 0.217 millimeter. On the ventral of the ninth and tenth abdominal segments there appears a pair of r mentary prolegs, so closely associated with each other that they s almost as one. In the second instar these are so small that their prese is detected with difficulty, while in the third instar they may be s distinctly with the aid of a lens, or even with the naked eye if the la is attempting to crawl about.

Thus the three instars of this larva exhibit successive stages in t modification of the whole body, especially in the head and the prothor The first instar retains the typical condition of the buprestid larvae, wi the relatively large head retracted into the prothorax; while each of t following instars exhibits a modification of the relative size of the he and the prothorax, until, in the third instar, the mature larva seems have lost the characteristics of the wood-boring Buprestidae.



1

2

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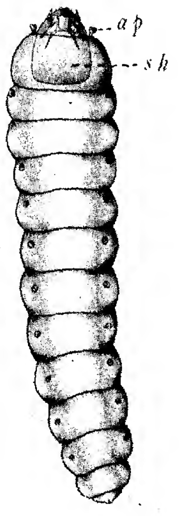
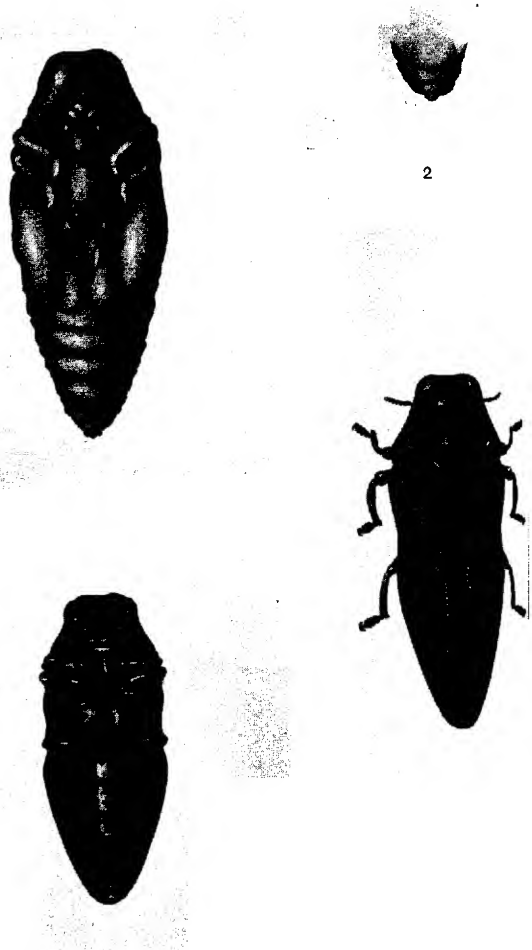


EGG AND LARVA OF TAPHROCERUS GRACILIS

on leaf. 2, First-instar larva. 3, Second-instar larva. 4, Third-instar larva (ap, prothoracic appendage; sh, chitinous shield of prothorax)

(Drawn by Helen A. Sanborn)



2

3

PUPA AND ADULT BEETLE OF TAPHROCERUS GRACILIS

1, Male pupa, ventral view. 2, Terminal segments of female pupa. 3, Adult beetle, ventral 4, Adult beetle, dorsal view

(Drawn by Helen A. Sanborn)

The pupa

The pupa resembles the adult in form and shape, but, unlike the pupae of the wood-boring buprestids that have been examined, it is encased in hard chitin. All the appendages are so closely appressed to the body that the chitinous covering is continuous over the whole surface, with only convexities and depressions marking the position of the appendages. The prothoracic leg, like the others, is so folded that the tibia is posterior to the femur, which, as may be seen from a comparison of the figures (Plate II, 1 and 3), is quite different from the condition in the adult, in which the prothoracic leg is folded anteriorly.

The sexes may be distinguished, with the aid of a lens, by the terminal abdominal segment of the pupa, which in the male is divided into three longitudinal parts (Plate II, 1 and 2). Also, the female is slightly larger than the male, altho this character is not sufficiently pronounced to be dependable.

LIFE HISTORY

Oviposition

The eggs are laid on the leaves of the flood-plain bulrush (*Scirpus fluviatilis*) from the middle of June until the middle of July. In the vicinity of Ithaca, New York, in 1915 and 1916, the majority of the eggs were laid between June 20 and July 10.

The female beetle selects the place for oviposition after careful inspection of the lower side of the leaves, sometimes spending six minutes or more in walking back and forth from the tip to the base of a leaf. Sometimes the leaf examined seems to be entirely unsuited for oviposition, and the beetle will then go to another leaf or even to another plant. Usually the beetle soon finds a place that seems favorable, thrusts out its ovipositor, and moves it back and forth like a paintbrush over the leaf surface. From five to seven pellets of excrement are usually placed on the egg during or immediately after oviposition. These adhere to the moist surface of the egg and may remain for several days. Three or four minutes is the usual length of time for the process of oviposition, altho it has been observed to take as long as five and one-half minutes.

As soon as one egg has been deposited, the beetle may go to another leaf or to another plant and repeat the process. One female observed completed ovipositing on one plant at 12.32 p.m. and began to oviposit on another plant at 12.38. Just how many eggs one female is capable of laying has not been ascertained, for it is very difficult to follow the small beetles in their rapid flight amidst the vegetation. About noon on a warm, sunny day was found to be the most favorable time to observe oviposition.

As many as four or five eggs have been found on a single leaf, but one or two is the average number. During August of 1915, scarcely a plant could be found among the almost pure growth of *Scirpus fluviatilis* at the head of Cayuga Lake, which did not have at least one egg on one or more of its leaves. The eggs were found anywhere from the tip to the base of the leaf, with the greater number slightly nearer the tip than the base and about midway between the midrib and the margin of the leaf.

The eggs assume a whitish appearance about ten minutes after oviposition, and gradually turn black, until, after forty-eight hours, they are a shiny black, as previously stated. Owing to the crescentic shape of the developing embryo, the eggs may appear to be slightly larger on one side than on the other. Under ordinary conditions they hatch in about ten days.

Emergence of the larva

The larva emerges from the egg by eating its way thru the side adhering to the leaf, and passes directly into the leaf tissue without exposing itself to the exterior. The fact that the egg is firmly attached to the leaf makes it possible for the larva to obtain leverage as it eats thru the leaf epidermis. The longitudinal veins of the leaf are just far enough apart to allow the flattened head and thorax of the larva to pass between them and permit it to enter the parenchyma, where it is surrounded by an abundant food supply.

The course taken by the larva as it first begins to mine its way about in the leaf seems to vary. It begins eating at once, forming a blotch mine which gives the leaf a blistered appearance. Some larvae begin to mine toward the tip of the leaf, while others mine on the side toward the base of the leaf. In either case the burrow assumes an area of three or four square millimeters in about twenty-four hours, and then the larva proceeds to mine toward the opposite end of the leaf. Ordinarily this alternating between one end of the mine and the other seems to continue until the larva has reached maturity.

An egg that was kept under constant observation hatched on June 13 and the larva made a mine, two square millimeters in extent, toward the base of the leaf the first day. By July 7 the mine measured 4 by 6 millimeters, the major part of which was toward the tip of the leaf. On July 17 the mine measured 4 by 55 millimeters, 35 millimeters of the length being toward the tip of the leaf. In three more days the burrow was complete, measuring 98 millimeters in length, and extending 35 millimeters toward the tip of the leaf and 63 millimeters toward the base.

The completed burrows were found to vary in length from 62 to 17 millimeters, and they usually extended from the midrib to the margin of the leaf, often causing the leaf to become rolled. A few leaves were found in which the larva had crossed the midrib and continued its mine on the other side. Others were found in which as many as four larvae working in the same leaf, had united their mines into one, and all the larvae had matured successfully.

In feeding, the larva devours all of the tissue between the two layers of the leaf epidermis. Its body, including the prothorax, remains stationary while its head moves from side to side until all the tissue within reach has been eaten. Then the prothorax is crowded forward, forcing the two layers of epidermis apart. From the new position, the process of eating all the tissue within reach is repeated. In holding the prothorax in place and in moving it forward to a new position, the small appendages of this part of the body seem to be used very much like ordinary pro-thoracic legs.

At the end of each instar the larva evidently returns to the central part of the mine to molt, for it is here that all of the castings, and eventually

the pupae, are to be found. The exact length of each instar has not been determined. It seems probable that the instars vary, for larvae of the different instars have been found at widely different times and in burrows of various lengths. The length of the larval stage varies from about three to four or more weeks, and the greatest amount of the mining is done during the last three or four days of feeding, as is shown by the measurements given in a preceding paragraph.

When the feeding has ceased, the larva measures seven or eight millimeters in length and appears rather plump. At this time it crawls to the more spacious central part of the mine, where, surrounded by an accumulation of dried pellets of excrement, it undergoes metamorphosis.

The pupa

The pupa as it first emerges from the larval skin, is soft and white. In the course of a few hours, the outer covering becomes hard and brown. In the laboratory this was observed to take place within about twelve hours, and field observations indicate that this is about the average length of time. No motion has ever been observed in the pupa from shortly after the time that it emerged from the larval skin until the emergence of the adult. Under laboratory conditions the pupal stage lasts ten days, which probably is a fair representation of the normal.

In order to permit the emergence of the adult, the pupal skin breaks along the median line from the anterior margin of the prosternum, on the ventral side, over the head and prothorax to the anterior margin of the mesotergum. As the adult appendages are drawn out, the chitinous covering of the pupa ruptures along the impressed lines which outline the wings and legs and the thoracic segments, making the emergence of the adult less difficult.

The adult beetle

Within a few days after emergence (which at Ithaca, New York, takes place about the second week in August), the adult beetles start feeding on the tender top shoots of the flood-plain bulrush. On warm, sunny days they may be seen feeding on the edges of the leaves, cutting little notches which are sometimes so deep that they cause the leaves to bend over. The beetles fly very rapidly, but they seldom seem to have occasion to fly about; several beetles have been observed to remain feeding on the same plant for more than two hours. Much of the time they walk about examining the edges of the leaves, apparently to no purpose.

They often continue to feed on warm autumn days even after the first frosts, which do not seriously injure the bulrush. At Ithaca in 1915 the beetles were abundant as late as October 14. In 1916, at the University of Minnesota, two beetles remained on bulrushes in a cage until October 5.

The hibernation of the beetles is as yet an unsolved problem. From the time of their disappearance from the leaves of the bulrush in the fall until they reappear on the new leaves in the spring, no trace of them has been found. The fact that the beetles have never been found in any part of the plants late in the fall or during the winter, together with the fact that the areas on which *Scirpus fluviatilis* grows are completely

flooded very early in the spring, makes it seem very probable that th beetles migrate in the fall to higher land for the purpose of hibernation.

The number of beetles found after the period of frost in the fall, gradu ally decreases until no more are to be found either on the plants or in th débris covering the ground. After severe frosts beetles have been found both in New York and in Minnesota, which were in a semi-dormani condition. These are always in the crevices of the leaf axils, and wher dislodged they fall to the ground, apparently too numb and helpless tc escape.

Large quantities of Scirpus and of the débris covering the ground have been taken to the laboratory and examined with the greatest care, but nothing more than a few fragments of dead beetles has ever been found. Since much of the Scirpus grows in several inches or even a foot of water, it would seem that there is little possibility of the beetles' falling from the plants in the autumn, spending the winter wherever they fell, and in some way surviving the spring floods.

One cannot help asking what becomes of the beetles that linger late in the fall and drop to the débris under the plants when dislodged. Are these beetles lost in the spring floods, and do only the beetles that migrated earlier in the fall survive the winter? Or do even these late beetles, as seems to be indicated by a cage experiment, revive in the warmth of some later autumn days and migrate to suitable winter quarters? As yet the assumption that the beetles migrate at all is not proved, altho the circumstantial evidence makes it seem to be a safe assumption. Certain other buprestid beetles hibernate as adults in the pupal cells, but none are i known to migrate to winter quarters (Burke, 1920; Knull, 1920 and 19:

ECOLOGY

Food plants

The questions of geographic and local distribution are bound up in food-plant relations, for no amount of searching has detected this bee anywhere except on the flood-plain bulrush, *Scirpus fluviatilis*. So as is known, the distribution of *Taphrocerus gracilis* is more limited th that of *Scirpus fluviatilis*, which occurs thruout northeastern and cent United States. Even isolated patches of the bulrush have their popu tion of beetles in central New York and southern Minnesota, while no were found in Lake County, Minnesota.

Blatchley (1910) states that *Taphrocerus gracilis* occurs on buttonbu (*Cephalanthus occidentalis*), but careful searching in various parts of N York has failed to discover it on this shrub. Since the beetles do som times light on other plants than *Scirpus fluviatilis*, it is possible that th may be taken occasionally on buttonbush.

Parasites

Egg parasites are the most abundant of all the parasites of this beetl A small braconid has been found to have parasitized as many as sevent per cent of the eggs toward the close of the season. These parasite usually occur two in an egg, but occasionally one is found and four ar not infrequent. The life history of this braconid has not been worke

it completely. At the time of its emergence, a round hole is made in ie top of the beetle egg thru which the adult parasites emerge.

A larval parasite, only three specimens of which were seen, was found the mines of the beetle larvae. This was an external parasite, deterined by Dr. A. A. Girault as a new species (*Achysocharis donna*). Other rval parasites that were found have not yet been determined.

Behavior of the beetles

Temperature seems to be a factor strongly influencing the behavior *Taphrocerus gracilis*. Observations have shown that on a warm, nny day the beetles are actively feeding on the leaves of the bulrush, d that they fly quickly when disturbed. On a cold day, and especially rly in the morning, the beetles are inactive and are found in the crevices tween the bases of the leaves and the stalk of the bulrush, and they tract their legs and fall back into the crevices when disturbed.

In the field experiments, the beetles were approached when on the lrushes, and if they did not respond in some way a pair of forceps was ved near them. They would then either retract their appendages and l, fly away, or cling to the leaves. If they clung to the leaves, they re mechanically stimulated by touching with the forceps until they re forced to contract and fall, or to fly away. Sometimes all three actions would be obtained from a single individual. At first it would ng to the leaf; when further stimulated it would retract its appendages d start to fall, and then it would begin to fly. Such a complex response s common at temperatures between 19° and 20° C. It was as if the etles were slightly torpid and the beginning of flight was delayed by slowness of response. If the beetles were in such a position on the ves that they fell into the crevices at the base, flight never began at If they were out near the tips of the leaves and actually fell, flight ght begin before they reached the water, at the above-named temperaes. At lower temperatures the beetles were rarely found out near the s of the leaves, but when they were they would fall great distances hout flying at all.

summary of 190 experiments in which different beetles were used for h experiment, except possibly when the same beetle was accidentally with on different plants, is given in table 1:

BLE 1. REACTIONS OF THREE LOTS OF BEETLES TO MECHANICAL STIMULATION AT DIFFERENT TEMPERATURES

(Numbers of beetles in respective lots, 27, 33, and 130)

Reaction	Temperature (degrees centigrade)		
	Below 19°	From 19° to 20°	From 20° to 30°
t that contracted	89	39	40
t that flew	4	54	77
t that clung to leaves	18	54	22

In table 2 are contained the results of a series of experiments whic began on June 30, 1917, before sunrise, while the temperature was low

TABLE 2. REACTIONS OF 82 BEETLES TO MECHANICAL STIMULATION
(Tested in succession as indicated, with rising temperature)

Number of beetles that			Temperature	Number of beetles that			Temperatur
Con-tracted	Flew	Clung		Con-tracted	Flew	Clung	
1			14.5°C.		1		19.5°
1		1			1	1	
1*					1		
1		1			1		
1					1		
1					1		
1*				1			
1				1	1	1	
1				1		1	
1*				1		1	
1					1	1	
1			15.5°		1	1	
1					1	1	
1				1		1	20.5°
1				1		1	
1		1			1	1	
1		1			1		
1		1			1		
1†		1	18.5°	1*		1	24°
1					1	1	
	1				1		
1					1	1	
1					1	1	
		1			1	1	
		1			1	1	
1				1		1	
		1	19°		1	1	
		1			1		
	1				1		
1					1		
		1			1		
1					1		
	1				1		
1		1			1		
	1				1		
1		1		1	1		
	1				1		
1		1			1		
1		1					
1		1					
	1						
	1						
	1						
	1	1					

* Beetle fell into water.
† Direct light from the rising sun began to fall on the plants at this time.

l continued until the sun was well up in the sky and the temperature l risen to 24° C. The time is not given, for the beetles were tested as y were found by searching about among the bulrushes.

t is to be noted from table 2 that there was a gradual change of the ponse until the beetles all responded by flying. July 7, 1917, was a windy and the bulrushes were being blown about, and in 46 experiments on t day, 35 beetles responded by flying, 10 by clinging, and 19 by con-cting. In this case, the proportion that contracted was unusually h, for the temperature varied from 27.5° C. at 10.15 a.m. to 30° C. noon. Of the 19 beetles that retracted their appendages, 6 first clung he leaves and had to be dislodged with the forceps when they fell.

This change of response, correlated with a rising temperature, was so rked that laboratory experiments were used to verify the field observa-is. The beetles were placed in a jar containing bulrushes and sur-nded by warm water. At a temperature of 30° C., they were very ve, responded positively to light, and when disturbed took to their gs or clung tenaciously to anything that came in contact with them. en cracked ice was substituted for the warm water and the temperature lowered to 15° C., the beetles became much less active and responded atively to light, which resulted in their retirement to secluded places t as the crevices in the axils of the leaves; and when disturbed, they acted their appendages and allowed themselves to fall instead of taking heir wings as with the higher temperature.

CORRELATION OF STRUCTURE AND HABITS

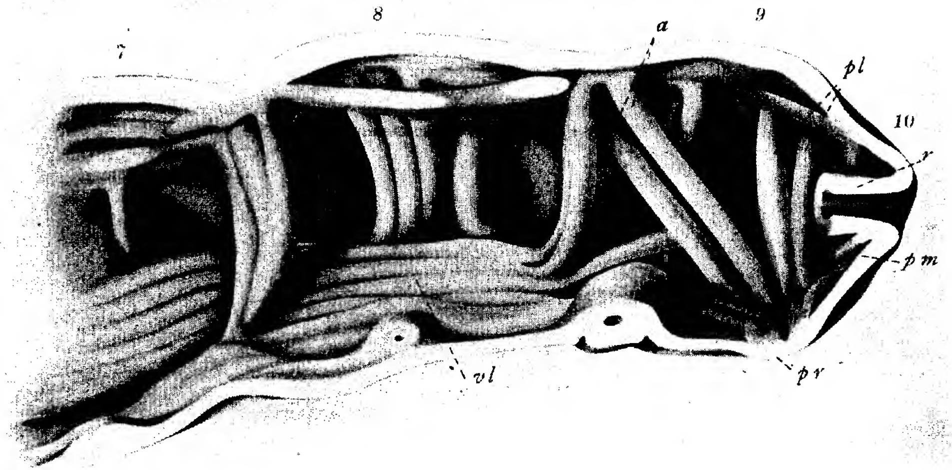
rom the field observations and the laboratory experiments described he preceding paragraphs, one may correlate the retractile appendages e beetles with their reactions. When the temperature is low, and the tive beetles, by reason of their negative reaction to light, are at the of the leaf, any disturbance will cause them to retract their appen-s and fall into the crevice between the leaf and the stem. Attempts emove a beetle will only serve to push the leaf sheath away from the of the plant and allow the smooth, flat body of the beetle to slide ler down into the crevice.

n warm, sunny days the active beetles are out on the foliage, in accord-with their positive response to light. When disturbed, they take to wings and escape, whereas if they responded as they do in a low erature, they would in many cases fall into the water.

e abdominal prolegs, or ambulatory ampullae,[2] of the larva may be idered as a structure correlated with its habits. Unlike its wood-g ancestors, the larva of this species crawls about in a mine between upper and lower layers of the leaf epidermis. The mine, as previously d, often appears as a blister with a spacious interior, in which the larva d be unable to move about without some organ of locomotion.

model of the posterior end of the abdomen was made, in order to mine what modifications have taken place in the development of apparently new structure. As shown by the drawings (Plates III IV), which represent, respectively, the right side and a part of the

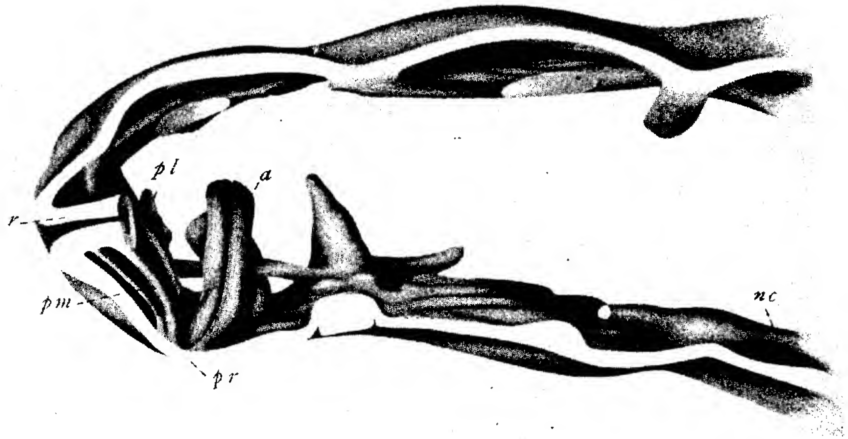
[2] *Ambulatory ampullae* is the name given by Craighead (1915) to somewhat analogous structures on ... larvae.



RECONSTRUCTION OF RIGHT HALF OF TERMINAL ABDOMINAL SEGMENTS OF LARVA, MEDIAN VIEW

a, Anterior group of muscles to proleg; pl, posterio-lateral group of muscles to proleg; pm, posterio-median group of muscles to proleg. pr, Proleg. r, Rectum. vl, Ventral layer of abdominal muscles. 7, 8, 9, 10, Abdominal segments

(Drawn by Helen A. Sanborn)



RECONSTRUCTION OF PART OF LEFT SIDE OF TERMINAL ABDOMINAL SEGMENTS OF LARVA, MEDIAN VIEW
nc, Nerve cord. Other symbols as for Plate III
(Drawn by Helen A. Sanborn)

left side of the larva, the prolegs are situated between the ninth and tenth segments. The model represents the right side of the larva as so contracted that the ventral longitudinal muscles of the left side are relaxed and the proleg of that side is near the midline. The oblique position of the ventral longitudinal muscles is such that when those on one side are contracted and those on the other side are relaxed, the end of the abdomen is turned toward the contracted side and the proleg of that side is lateral to the midline while the proleg of the relaxed side approaches the midline.

There are three groups of muscles entering each proleg. As shown in Plate III, the anterior group, consisting of three muscles, originates on the dorsal wall between the eighth and ninth segments and is inserted in the anterior part of the proleg. The posterio-lateral group of two muscles originates on the dorsal wall in the region between the ninth and tenth segments, and is inserted in the proleg posterior to the first group. The posterio-median muscles are very small, and originate on the wall of the rectum and pass to the proleg.

The posterio-lateral group of muscles evidently represents the dorso-ventral muscles which occur normally between the segments, as may be seen in the seventh and eighth segments in the model. The posterio-median group of muscles, from the rectum, are, no doubt, the equivalent of the muscles passing from the rectum to the dorsal wall of the abdomen and are enlarged slightly in connection with their function in the proleg. The homology of the anterior group of muscles is much more difficult to determine. It probably is a modification of an oblique group of muscles which are present in the terminal segments of the abdomen but which are not represented in the other segments.

The prothoracic appendages are also worthy of mention as structures correlated with the habits of the larva. These structures, as has been stated, assist in holding the prothorax in place while the larva is feeding. They are supplied with muscles, and are covered with a thin, spinous layer of chitin. All evidence seems to point to the fact that they are new structures somewhat analogous to the prolegs in other larvae.

SUMMARY

This beetle is of special interest because, altho it belongs to the Buprestidae, a famous family of wood-borers, it mines in the leaves of a bulrush *Scirpus fluviatilis*.

The larval life is only about three or four weeks in duration. The pupal stage lasts ten days, and the adults spend the remainder of the season feeding on the foliage of the food plant, *Scirpus fluviatilis*. The method of hibernation is not known, but it seems probable that the adult beetles migrate to the upland to pass the winter, since none have been found in their usual haunts between late fall and early spring.

The beetles are restricted to one food plant, *Scirpus fluviatilis*, and are found only on that plant except when they have accidentally alighted on other plants, which they leave immediately.

During the first instar the larva is structurally much like that of the wood-boring Buprestidae, but in the subsequent instars it resembles other leaf-mining larvae. It has a pair of appendages developed on the

thorax which assist it while feeding, and a pair of ambulatory ampullae, prolegs, developed on the abdomen which enable it to crawl about within mine in the leaf.

The adult beetles are greatly influenced by temperature, and this behav- complex seems to be very important. In high temperature and strong it, they are very active and take to their wings or cling to the leaves en disturbed; but in low temperature, they are inactive, retract their pendages, and drop into the crevices at the base of the leaves when turbed. This seems to be a protective measure, for the beetles would many cases fall into the water if they retracted their appendages and opped from the plant while actively feeding on warm days. The fact t they are near the base of the leaves on cold days seems to be due to ir negative response to light in low temperature, while their occurrence the foliage on warm days seems to be due to their positive response light in high temperature.

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